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SCIENTIFIC AGRICULTURE

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SCIENTIFIC AGRICULTURE

VOLUME 32 DECEMBER, 1952 No. 12

PRESERVING RAW MILK WITH VARIOUS ANTIBIOTIC PREPARATIONS

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[Received for publication April 21, 1952]

INTRODUCTION

Among the numerous aspects of swine husbandry, the feeding of suckling pigs away from the sow remains an important economic problem. Occasionally the sow secretes insufficient milk at farrowing time to adequately supply her litter owing to some degree of lactation failure or to the birth of a large litter. Hand-feeding of piglets is inconvenient and expensive, but, if a self-feeding unit were employed from which the piglets could freely obtain warm milk, this problem would be near solution. It would be necessary, however, in a unit such as this, to add some preservative to the milk in order to keep it wholesome over an extended period of time (12-24 hours) in view of the holding temperature involved (37° C.).

Various commercial antibiotics are at present being added to swine-feeds in order to improve their nutritional value. Most of these preparations are known to have active bacteriostatic properties. Consequently, a series of investigations was undertaken to determine the value of a number of these compounds with particular regard to their preservative effect on raw milk held at body temperature. Throughout these trials, economy and facility of preservation were given consideration with the ultimate aim of developing a practical preparation for routine use.

REVIEW OF THE LITERATURE

A number of investigators have studied the preservative action of antibiotics in foods. Curran and Evans (6) described the sporicidal activity of penicillin and suggested that it could be used in the food processing industry. Anderson and Michener (3) used a combination of heat and 10 p.p.m. of subtilin to preserve peas, asparagus, corn, beans, potatoes, tomato juice and milk. Trials conducted with fish led Tarr *et al.* (13) to conclude that aureomycin and terramycin were very effective, while subtilin, penicillin and streptomycin showed only feeble preservative action. Williams and Campbell (16) found subtilin to be ineffective for the complete destruction of several thermophilic flat-sour bacteria, whereas Adams *et al.* (1) reported that 100 to 1000 p.p.m. of subtilin delayed spoilage of heated comminuted beef for 1½ months.

Most of the investigations on antibiotics in milk (4, 5, 7, 8, 15) have been concerned with the inhibitory effect of mastitis curatives on cheese starters. Poetschke (12), however, used streptomycin as a preservative of

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human milk. Two milligrams of streptomycin per 100 ml. of milk was quite inhibitive to organisms without impairing in any way the nutritional or physiological qualities of the milk.

EXPERIMENTAL METHODS

The raw milk samples used during these trials were obtained from the University herd and from a local commercial dairy plant. Precautions were taken to accept only fresh milk from animals having no history of antibiotic therapy. After thoroughly mixing and tempering the milk to 37° C., it was divided into a number of 100 ml. portions, each of which was placed in a clean Erlenmeyer flask. Appropriate aqueous dilutions of the test antibiotic were added to the flasks and were dispersed by shaking. The flasks were kept at 37° C. in a laboratory incubator. Initially and at regular intervals throughout the test period representative samples were aseptically removed for bacteriological and chemical analyses.

Increase in titratable acidity was the original criterion for estimating microbial activity in the raw milk. It was assumed that the particular preservative agent or concentration of that agent was ineffective if it did not delay acid production. When any given compound proved satisfactory in so far as acid production was concerned, its bacteriostatic efficiency was confirmed by standard plate counts on tryptone glucose extract agar. Both chemical and bacteriological tests were performed according to Standard Methods for the Examination of Dairy Products (2).

Since raw milk contains many types of microörganisms, not all of which are susceptible to any given bacteriostatic agent, a variety of antibiotics and sulfa compounds were tested. Furthermore it was considered advisable to assay the preservative action of various antibiotic feed supplements. The following preparations and combinations of these preparations were tested: Aurofac, terramycin, streptomycin, sulfathalidine, sulfaguanidine, penicillin, aureomycin, APF (Vitamin B_{12} and antibiotic feed supplement) and ustilagic acid.*

RESULTS

During preliminary runs two of the compounds were judged to be inadequate as preserving agents. Animal Protein Factor (APF) had no significant bacteriostatic effect, even in concentrations as high as 2.5 per cent. Similarly, concentrations up to 1000 p.p.m. of ustilagic acid in aqueous solution were ineffective. When this compound was added in alcoholic solution, a certain degree of microbial inhibition was observed, but subsequent trials revealed that this effect was due to the alcohol rather than the antibiotic.

Figure 1 illustrates the relative bacteriostatic properties of several commercial antibiotics. The raw milk in this case was of poor quality, and showed a rapid increase in acidity. Bacitracin delayed acid production for

* *Aurofac*—vitamin B_{12} and antibiotic feed supplement, 1.8 gm. aureomycin-HCl per lb. Courtesy Lederle Laboratories Division, Pearl River, N.Y.
Terramycin—terramycin-HCl. Courtesy Chas. Pfizer & Co. Inc., Chicago, Ill.
Streptomycin—streptomycin-$CaCl_2$ complex. Courtesy Merck & Co. Ltd., Montreal, Que.
Sulfathalidine—veterinary grade. Courtesy Sharp & Dohme, Philadelphia, Pa.
Penicillin—crude procaine penicillin G. Courtesy Merck & Co. Ltd., Montreal, Que.
Sulfaguanidine—purified. Courtesy Naugatuck Chemicals, Elmira, Ont.
Aureomycin—aureomycin-HCl. Courtesy Lederle Laboratories Division, Pearl River, N.Y.
APF—vitamin B_{12} and antibiotic feed supplement, 2 gm. procaine penicillin G per lb. Courtesy Merck & Co. Ltd., Montreal, Que.
Ustilagic acid—prepared by Prairie Regional Laboratory, N.R.C., Saskatoon. Courtesy R. H. Haskins

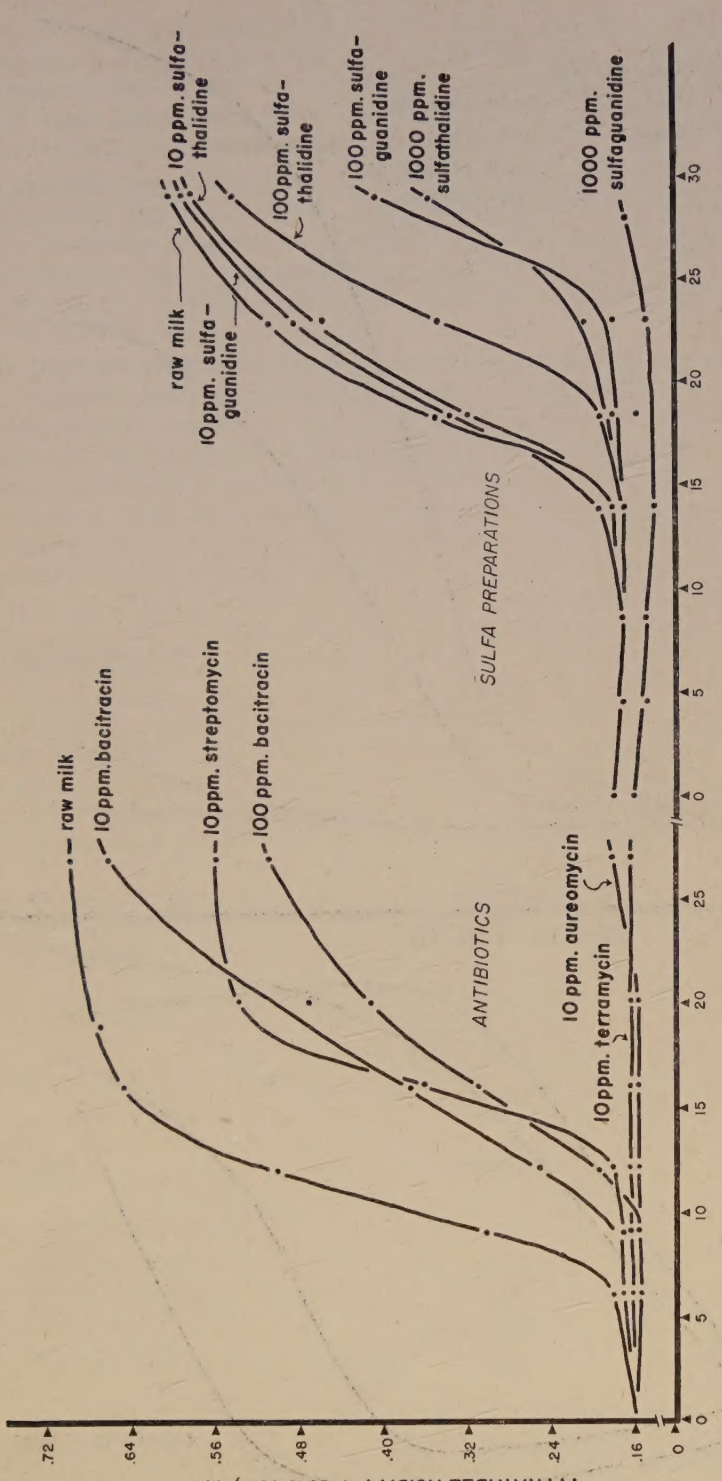

FIGURE 1. Effect of various antibiotics and sulfa preparations on acid production in raw milk held at 37° C.

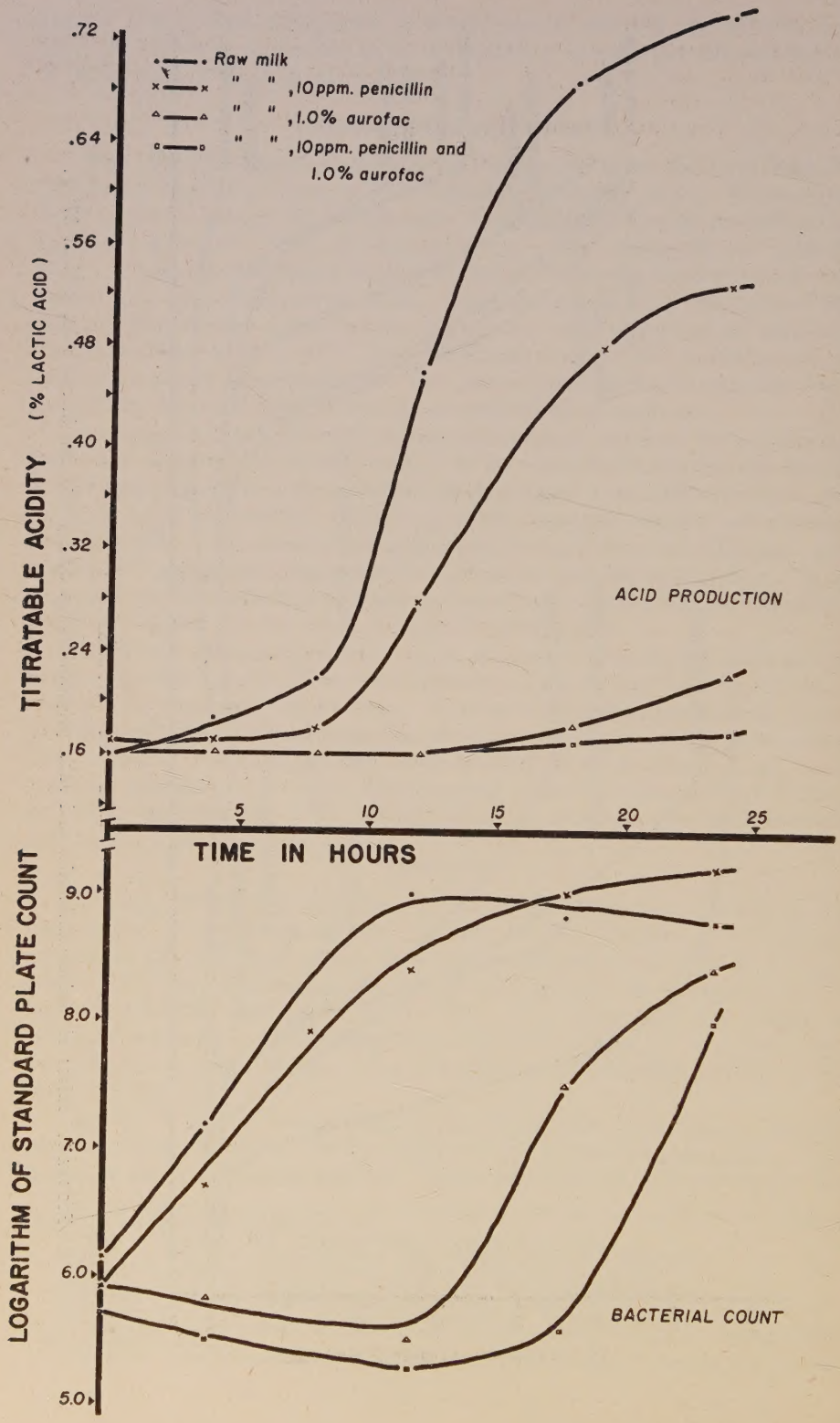

FIGURE 2. Effect of penicillin and aurofac, singly and together, on acid production and bacterial count in raw milk held at 37° C.

at least 9 hours, and slightly inhibited this process once it had started. Ten p.p.m. of streptomycin were more effective than a similar quantity of bacitracin, but neither was as efficient as terramycin or aureomycin. Both of these latter compounds preserved the milk for over 20 hours, a sufficiently long time to render them useful for the project under discussion.

Two sulfa preparations also showed promise as possible preservatives. Sulfaguanidine was more effective than sulfathalidine, but the effectiveness of either compound was directly proportional to its concentration. These results, however, may be misleading because the raw milk employed during this trial was of unusually good quality, showing no increase in acidity for 14 hours at 37° C.

In Figure 2 are shown the results obtained by combining two preparations, neither of which was satisfactory alone. The acidity curves illustrate the rapid deterioration of raw milk, the slight inhibitory effect of 10 p.p.m. of penicillin, the more pronounced effect of 1.0 per cent aurofac, and the excellent preservation accomplished by combining the two. The slight rise in acidity which was observed after 18 hours in the aurofac sample was not truly indicative of the deterioration which had occurred, since a definite putrefactive change had taken place by this time.

This phenomenon is further illustrated by the bacterial counts. The growth in the raw and the penicillin-treated samples occurred in a normal fashion with only slight inhibition effected by penicillin. The bacterial count of the aurofac sample decreased during the initial 12 hours, but this was followed by a sharp increase, so that by 18 hours the count had increased 40-fold from the original. However, the sample which contained both aurofac and penicillin showed no significant increase in numbers until after 18 hours. Also worthy of mention are the casual observations that odour and consistency of milk thus preserved remained unchanged.

TABLE 1.—THE EFFECT OF VARIOUS ANTIBIOTIC PREPARATIONS ON ACID PRODUCTION AND BACTERIAL DEVELOPMENT IN RECONSTITUTED POWDERED MILK

Antibiotic	Acidity developed in 24 hr. (calc. as % lactic acid)	Standard plate count	
		Initial	After 24 hr.
		($\times 10^3$)	($\times 10^6$)
Raw control	+ 0.35	0.7	300
Reconstituted skimmilk[1]	+ 0.31	300	300
Reconstituted fortified skimmilk[2]—			
No antibiotic	+ 0.41	230	900
Terramycin (10 p.p.m.)	0.00	500	0.24
Aureomycin (10 p.p.m.)	− 0.01	43	0.30
Streptomycin (10 p.p.m.)	+ 0.74	400	800
Aurofac (1.0%)	+ 0.02	23	29
Penicillin (10 p.p.m.)	+ 0.26	110	300
Penicillin (10 p.p.m.) + Aurofac (1.0%)	− 0.02	22	0.24

[1] Reconstituted skimmilk: 10 gm. non-fat-dry-milk-solids, 100 ml. water.

[2] Reconstituted fortified skimmilk: 26.5 gm. non-fat-dry-milk-solids, 0.05 gm. feeding fish oil (1500A, 200D), 0.0363 gm. mineral mix, 100 ml. water. Mineral mixture: 100 gm. $FeSO_4 . 7H_2O$; 6 gm. $CuSO_4 . 5H_2O$; 4 gm. $CoCO_3$; 6 gm. $MnSO_4 . 7H_2O$; 10 mg. KI; 6 gm. $ZnSO_4 . 7H_2O$, and 15 gm. $MgSO_4 . 7H_2O$.

In one series of trials reconstituted milk-powder was used in place of fluid raw milk because milk-powder, fortified with appropriate minerals, vitamins and antibiotics, offers a number of advantages and is the basis of some commercial preparations.

The results obtained during tests on reconstituted milk powder are presented in Table 1. These data are in agreement with those reported for fluid raw milk.

The bacterial count of the aurofac sample increased significantly after 12 hours, whereas no corresponding increase could be detected in its acidity. Penicillin plus aurofac were more effective than either of the two used alone. Terramycin and aureomycin inhibited growth and acid production for approximately 24 hours, while streptomycin in the concentrations used was relatively ineffective.

DISCUSSION

In attempting to inhibit the development of a mixed microbial culture, such as is found in raw milk, consideration should be given to the different types of organisms present and to their respective susceptibilities to various bacteriostatic agents. Thus a suitable preservative should include compounds which inhibit both gram positive and gram negative organisms. Further consideration must be given to the cost and availability of these compounds, as well as, in this case, to their usefulness in animal nutrition. The results reported in this paper have met some of the above requirements. A mixture of penicillin and aurofac was able to preserve raw and reconstituted milk for 24 hours at 37° C. These compounds are relatively inexpensive, commercially available, and are being widely used in feeding live stock and poultry. Terramycin and aureomycin were also effective in very small concentrations.

It must be recognized that these trials were not carried out under farm conditions and that the concentrations of antibiotics found effective in these experiments may be inadequate for practical application. Final evaluation of milk formulas thus preserved must await actual feeding tests. It is worth noting that Luther *et al.* (9) have reported good results in baby pigs fed a terramycin-treated milk substitute. Others (10, 11, 14) have demonstrated favourable responses in baby pigs fed antibiotics.

SUMMARY

It was found that aureomycin and terramycin added to raw milk in the order of 10 p.p.m. effectively inhibited both acid production and bacterial growth for 20 hours at 37° C. Sulfaguanidine and sulfathalidine showed good preservative action when 1000 p.p.m. were used.

Penicillin and aurofac, though relatively ineffective separately, exerted marked bacteriostatic action when added in combination in concentrations of 10 p.p.m. and 1.0 per cent, respectively.

The findings are discussed in the light of recent reports on the use of milk substitutes for self-feeding baby pigs.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance rendered by Merck & Co. Ltd., Lederle Laboratories, Chas. Pfizer & Co. Inc., Sharp and Dohme, Naugatuck Chemicals, and the Prairie Regional Laboratory, Saskatoon, for generously supplying antibiotics and sulfa preparations. Credit is due W. C. Lawton, formerly graduate assistant, Department of Dairying, University of Saskatchewan, for bacteriological work in the early phases of the project.

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HAY AND CRUDE PROTEIN YIELDS, WINTER HARDINESS AND WILT RESISTANCE OF ALFALFA VARIETIES[1]

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[Received for publication December 28, 1951]

The question of new alfalfa varieties is an important one today in Western Canada. In recent years several new varieties have been developed in Canada and the United States and licensed for sale. Although some of these new varieties are adapted over a wide area, the use of others must remain confined to the locality of their origin. In view of the constant change in the alfalfa variety picture it is important that local Experimental Stations and Universities test these varieties so that the farmer may be told of their relative merits. It was for this purpose that two variety trials were seeded in 1945 at Swift Current, Saskatchewan, one on irrigated land and the other on dryland. The varieties were compared for hay yield, protein content, winter hardiness and wilt resistance.

MATERIALS AND METHODS

The varieties included in both tests were Ladak, Grimm, Ranger, Buffalo, Viking, Rhizoma, Ferax, Canauto and a local selection from Ladak designated as Ladak Sc3465. The origins and descriptions of the first four varieties are given by Westover (7); that of Viking is given in a Canada Department of Agriculture publication (3); that of Rhizoma has been presented by Nilan (6), and that of Ferax by Fryer (4). According to Armstrong[5], Canauto originated from eight self-tripping plants selected out of Grimm Saskatchewan No. 666. These were synthesized into a strain by repeated crossing. The variety represents an attempt to improve seed production independent of bee activity.

The irrigated test was seeded in May, 1945, on an alluvial clay loam soil. A complete randomized block design with four replications was used. Plots measured 10 feet wide and 20 feet long, and were seeded at a rate of 15 pounds per acre in 6-inch rows. Dry conditions following seeding necessitated irrigating, and excellent stands of all varieties resulted. One other irrigation was given during the first year and the test was mowed once for weed control. Subsequently, three to five irrigations were applied per season, depending on rainfall. One of these was a fall irrigation. Two cuttings were made each season, the first about the end of June, and the second around August 10.

In the late summer of 1950, the concluding year for the irrigated test, all plots were rated for degree of bacterial wilt infection. Ten plants, dug up at 18-inch intervals along the centre row of each plot, were examined. A rating of zero represented complete freedom from visual symptoms of

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TABLE 1.—WEATHER RECORDS AT SWIFT CURRENT, SASKATCHEWAN, 1945–1950*

Year	Annual Precipitation in inches	Evaporation in inches (May to Sept.)	Precipitation Evaporation Ratio	Frost-Free Period (32° F.) Days	Mean Temperature			Winter Snowfall in Inches
					Nov.–Mar.	Apr.–Oct.	Annual	
1945	12.14	27.07	0.46	111	13.3	51.3	36.6	26.0
1946	14.36	26.07	0.55	103	12.7	53.7	38.9	32.3
1947	11.69	28.20	0.42	117	13.6	54.6	38.4	36.8
1948	12.25	28.05	0.44	103	10.3	55.2	38.2	28.5
1949	10.58	35.67	0.29	99	8.9	54.1	39.5	27.6
1950	16.27	26.04	0.62	87		51.2		
Average	(28 yr.) 13.28	(30 yr.) 29.21	0.46	(28 yr.) 106			(27 yr.) 38.4	

* Data supplied by Soil Research Laboratory, Swift Current, Saskatchewan.

wilt disease, while a rating of five indicated that plants were dead or practically destroyed by the disease. At the same time as wilt ratings were made, percentage stand was determined by visual estimation.

The dryland test was seeded in May, 1945, on Wood Mountain Loam soil in a complete randomized block design with four replications. The plots were 8 feet wide by 40 feet long and were seeded at a rate of 8 pounds per acre in rows one foot apart. Excellent stands of all varieties resulted. Only one cutting per season was possible on dryland, and this was harvested between June 20 and June 30.

Yield data were obtained from the dryland test for the years 1946, 1947 and 1948. Severe drought in the fall of 1948, rigorous conditions through the following winter and continued drought in the spring of 1949, combined to cause considerable killing of alfalfa. No further yields were harvested, but percentage winter injury was estimated in May, 1949. Relative stand, in per cent ground cover, was recorded on each plot in June, 1950, using a point sampling method developed at this Station (2), and based on a modification of Levy and Madden's method (5). Thirty-six points one inch apart were sampled at 10 sites on each plot, for a total of 360 points per plot.

Dry matter samples from both tests were analysed for total nitrogen by the Kjeldahl method (1). Crude protein content in per cent was calculated by multiplying total nitrogen by the factor 6.25. Crude protein yields per acre were calculated by multiplying per cent crude protein by the dry matter yield. Hay yields per acre were calculated on the basis of 12 per cent moisture.

CLIMATE

Climate at Swift Current is characterized by low annual precipitation, high evaporation, a relatively short frost-free period, sub-zero temperatures during winter and light snow cover. Table 1 presents selected weather records for the period of these two tests, together with certain 28-year averages.

The long cold winters and summer droughts form a combination of climatic conditions which are unfavourable for the survival of alfalfa on dryland. Low precipitation in the period following cutting of the hay crop results in a poor second growth. Thus, plants often go into winter without accumulating adequate root reserves. On irrigated areas, fall irrigation will produce a good regrowth, thus assuring sufficient root reserve for survival. Nevertheless, even under irrigation, climatic factors other than moisture are often severe enough to cause considerable winter killing.

Although over two feet of snow was recorded each year, there were periods during each winter when the plots were bare. Chinook winds often melted the snow, or strong winds blew it away. Thus, portions of the test areas were often bare of snow during the winter. This condition was more pronounced on the dryland than on the irrigated test area.

The severe drought prevailing in the summer of 1949 is indicated by the P/E ratio of 0.29 for that year as compared to the average of 0.46.

RESULTS

Irrigated Test

The hay yields, per cent protein content and protein yields are presented in Table 2.

TABLE 2.—FIVE-YEAR AVERAGE HAY AND PROTEIN YIELDS OF ALFALFA VARIETIES BY CUTTINGS UNDER IRRIGATION

Variety	First Cutting in June		Second Cutting in August		Annual	
	Hay tons/acre	Protein %	Hay tons/acre	Protein %	Hay tons/acre	Protein lb./acre
Ladak	2.22	18.2	1.68	19.8	3.90	1318
Grimm	2.28	18.0	1.62	19.0	3.90	1289
Viking	2.23	18.2	1.58	19.8	3.81	1300
Ladak Sc3465	2.21	18.7	1.58	19.8	3.79	1298
Rhizoma	2.05	19.5	1.64	21.0	3.69	1346
Ranger	2.00	17.0	1.58	19.1	3.58	1160
Ferax	1.87	18.9	1.51	19.5	3.38	1166
Buffalo	1.81	17.1	1.46	19.5	3.27	1079
Canauto	1.74	18.5	1.38	19.8	3.12	1079
L.S.D. (P = .05)		.09		1.0	0.25	94

Analysis of Variance

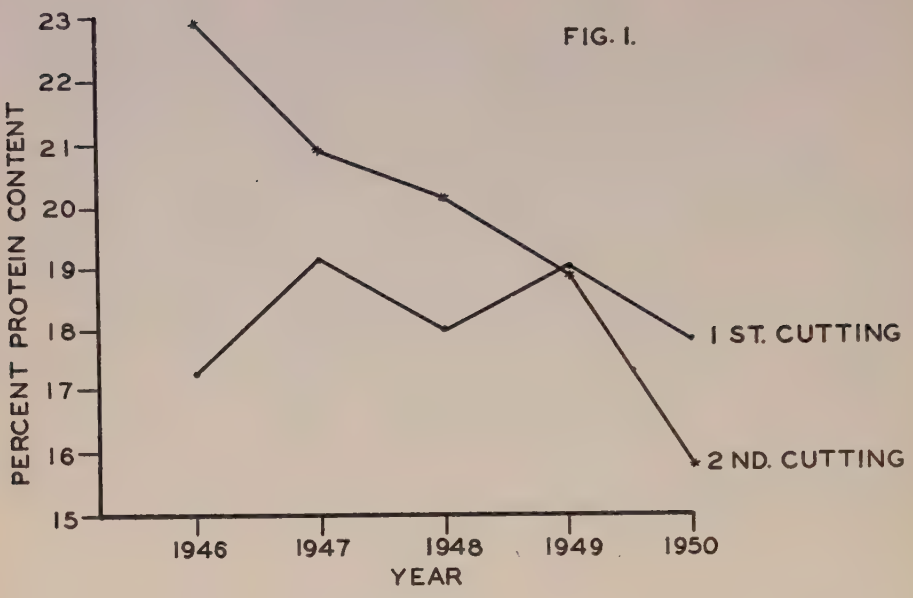
Source of Variation	Degrees of freedom	Mean Square			
		Hay yield	% Protein		Protein yield
			1st cut	2nd cut	
Varieties	8	1.65**	12.85**	6.84*	56.5**
Replicates	3	.39	1.50	.61	9.3
Var. × Rep. (Error A)	24	.16	1.87	2.40	5.5
Years	4	10.67**	28.45**	246.61**	4897.5**
Years × Varieties	32	.17	2.14	2.96**	72.5*
Remainder (Error B)	108	.13	1.76	1.47	42.7

* Significant at the 5% level.
** Significant at the 1% level.

TABLE 3.—BACTERIAL WILT INFECTION AND PERCENTAGE STAND OF ALFALFA VARIETIES ON THE IRRIGATED TEST, SEPTEMBER, 1950

Varieties	Bacterial Wilt Infection*	Visual estimate of stand in %	
		Actual	Data converted to P = $\sin^2\theta$
Ranger	0.23	93.7	80.1
Buffalo	0.53	85.0	68.8
Ladak Sc. 3465	1.25	71.2	58.1
Ladak	1.28	72.5	58.4
Viking	1.38	77.5	61.7
Canauto	2.02	68.7	56.3
Ferax	2.35	50.0	44.8
Grimm	2.52	78.7	62.7
Rhizoma	2.54	68.7	56.2
L.S.D. (P = .05)	0.87		12.3
Standard Error Mean %	19.7		7.2

* 0 rating represents freedom from wilt disease and 5 the most severe infection.



AVERAGE PERCENT PROTEIN CONTENT OF ALFALFA VARIETIES IN SUCCESSIVE YEARS

The two varieties, Grimm and Ladak, at present recommended in the Prairie Provinces of Canada, produced the greatest hay yields over a 5-year period. However, these yields were not significantly greater than those of Ladak Sc3465, Viking and Rhizoma. Ladak, considered to be a relatively poor yielder at the second cutting in many areas, did not show this characteristic at Swift Current. Ranger, although yielding less than Ladak during the first four years, produced the same quantity of hay in the fifth year when bacterial wilt began to reduce the stand of less resistant varieties. Ferax, Buffalo, and Canauto were considerably inferior in yield.

An outstanding characteristic of Rhizoma was its high protein content. It contained significantly more protein than any other variety and although placing fifth in hay yield it was at the top for protein yield. The significant variety year interaction for protein content at the second cutting may be due to small differences in maturity at harvest since the varieties were cut on the same day each year, about August 10.

An interesting observation was the reduction in protein content with the age of the stand of the second cutting. This is illustrated in Figure 1. The reason for this trend was not apparent but could be due to decline in nitrogen fertility of the soil. A nitrogen deficiency would tend to show up more in the second growth.

The extent of bacterial wilt infection and the estimated percentage stand of the varieties at the conclusion of the test in 1950 are shown in Table 3. The data clearly indicate the superior bacterial wilt resistance of Ranger and Buffalo over other varieties and the fact that Ladak and Viking are more resistant to the disease than Grimm, Ferax and Rhizoma.

To determine the relationship between wilt infection and percentage stand, the correlation coefficient was calculated using individual plot data. The value of $-.79$, significant at the 1 per cent level, indicates that 62 per cent of the reduction in stand could be attributed to the effects of bacterial wilt.

Dryland Test

The hay yields, per cent protein content and protein yields are recorded in Table 4.

Significant differences at the 5 per cent point between the three-year average yields of the varieties were not established by analysis of variance. However, Ladak significantly outyielded all varieties except Ladak Sc3465 and Rhizoma in 1947, the year of highest yields. Individual plot yields varied considerably and according to observations in the early spring, much of this variation could be attributed to differential snow accumulation on the plots. Rhizoma was outstanding in protein content.

The summer and fall of 1948 were extremely dry and consequently, none of the varieties made any regrowth after cutting. As a result, considerable killing occurred during the severe winter of 1948–49. The extent of winter injury, estimated in the spring of 1949, and the per cent ground cover, determined in 1950 by point sampling, are presented in Table 5.

TABLE 4.—THREE-YEAR AVERAGE HAY AND PROTEIN YIELDS OF ALFALFA VARIETIES ON DRYLAND

Varieties	Hay Yield ton/acre	% Protein Content	Protein Yield lb./acre
Ladak	.87	15.7	245
Ladak Sc3465	.76	16.3	218
Rhizoma	.73	17.6	233
Canauto	.69	17.1	204
Buffalo	.68	15.9	192
Viking	.67	16.5	196
Grimm	.62	16.7	185
Ranger	.56	16.4	160
Ferax	.51	17.0	155
L.S.D. (P = .05)	N.S.	.9	N.S.

Variance Analysis

Source of Variation	D.F.	Mean Square		
		Hay yield	% Protein	Protein yield
Varieties	8	.1393	4.3175*	10887
Replicates	3	.7209	.7750	61795
Var. × Reps. (Error A)	24	.0716	1.2814	6280
Years	2	2.9521*	26.1803*	231350*
Years × Varieties	16	.0621	.7438	4205
Remainder (Error B)	54	.0547	.7678	4622

* Significant at the 1 per cent level.

TABLE 5.—ESTIMATED WINTER INJURY TO ALFALFA VARIETIES DURING THE WINTER OF 1948–49 AND BASAL COVER IN 1950 ON DRYLAND

Varieties	Percentage winter injury 1948–49		Percentage Basal Cover—1950
	Actual	Data converted to $P = \text{Sin}^2\theta$	
Ladak	3.4	10.7	9.9
Ladak Sc3465	3.4	10.7	11.1
Viking	12.1	20.4	7.4
Ranger	12.1	20.4	5.3
Canauto	15.4	23.1	5.9
Rhizoma	15.8	23.4	7.6
Grimm	23.2	28.8	6.3
Ferax	27.6	31.7	4.3
Buffalo	33.2	35.2	3.1
L.S.D. (P = .05)		14.9	1.0
Standard Error Mean %		23.4	5.0

Ladak was the hardiest variety. A surprising result was the outstanding winter hardiness of Ranger, which compared favourably with Grimm, Viking and Rhizoma. The variability in winter injury between plots of the same variety was quite great as is indicated by the standard error of the mean. The data on per cent basal ground cover give a precise picture of the relative stand of the varieties in 1950. Ladak and Ladak Sc3465 had a significantly better stand than all other varieties. It was noted that Rhizoma , which suffered as much winter injury in the winter of 1948–49 as Canauto had a much better ground cover in 1950. This may be due to a slight crown spreading during the interval between estimation of winter injury in 1949 and point sampling in 1950. Although Rhizoma did not exhibit rhizomatous development at Swift Current, it did produce crowns that were wider than those of the other varieties included in the test.

The correlation coefficient, calculated on the basis of individual plot data, between winter injury, recorded in 1949 and per cent basal ground cover in 1950 was $-.84$, significant at the 1 per cent level. This indicated that 70 per cent of the reduction in stand was attributable to winter killing during the winter of 1948–49.

DISCUSSION

On the basis of results from the two experiments Ladak appears to be the most suitable variety for both irrigated land and dryland in Southwestern Saskatchewan. It produced the highest forage yields in both tests, was at the top for winter hardiness and possessed fair resistance to bacterial wilt. Viking and Ladak Sc3465 performed much like Ladak, but were not superior to it in any respect. Rhizoma and Grimm produced good forage yields, but both were damaged to a much greater extent by bacterial wilt on irrigated land than Ladak. Ranger and Buffalo were definitely more wilt resistant than other varieties in the test, but they yielded considerably less than Ladak. Of the two varieties, Ranger proved to be more winter hardy and under severe bacterial wilt conditions could be used in place of Ladak. Canauto and Ferax were the least desirable of the varieties tested.

The results from the analysis for crude protein content indicate that varieties differ considerably in this important factor of feed quality. Rhizoma contained significantly more protein than any other variety. This suggests a genetic basis for protein content and it should be possible to develop high or low protein strains by breeding.

SUMMARY

Results are reported from two alfalfa variety trials conducted at the Dominion Experimental Station, Swift Current, Saskatchewan, one on irrigated land and the other on dryland. Each trial included the varieties, Ladak, Grimm, Viking, Ferax, Canauto, Rhizoma, Ranger, Buffalo and the selection Ladak Sc3465. Data were obtained on hay yield, protein content, protein yield, winter hardiness and wilt resistance.

Ladak produced the greatest hay yield on both irrigated land and dryland. It suffered considerably less damage from bacterial wilt under irrigation than Grimm and was significantly hardier on dryland.

Ranger and Buffalo exhibited a high degree of wilt resistance, but on dryland Buffalo proved to be insufficiently winter hardy.

Rhizoma contained the highest amount of protein in both tests and on irrigated land it was the top variety in protein yield over the 5-year period.

It was concluded that Ladak is the best suited variety for both irrigated land and dryland in Southwestern Saskatchewan. On irrigated land where wilt infection may be severe Ranger would be satisfactory providing it was given careful management to minimize possible winter injury. No other varieties appeared to possess attributes which would warrant their use in preference to Ladak.

ACKNOWLEDGMENTS

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EFFECTS OF VARIOUS GROWING METHODS IN LEEK SEED PRODUCTION[1]

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INTRODUCTION

Leek grown for seed is a biennial crop, well suited to the climate of southern Vancouver Island where the long growing season and mild winters permit it to overwinter successfully in the field. As a consequence the greatest part of the Canadian production has been in this area. While a minor seed crop, particularly at the present time when Great Britain is unable to import because of financial restrictions, it represents a potential source of income to seed-growers. Whether the overseas seed-market will again open up for Canadian-grown leek seed will undoubtedly depend to a large extent upon the possibility of reducing production costs. Hence it is important to know the most efficient growing methods.

The first commercial planting of leek on southern Vancouver Island was made in 1928, seed being sown in a nursery-bed in the spring, and plants moved to the field in the fall. Webster (1) mentions that the transplanting method is preferred to sowing in the permanent row because of reduced weeding and thinning costs. While the transplanting is usually done in summer, some growers have practised fall transplanting because of more favourable soil moisture. No data have been available as to the merits of transplanting at different times or of plantings being allowed to stand to maturity where sown, that is, the seed-to-seed method.

Growers have frequently had difficulty in producing seed which will reach the 65 per cent germination required for sale under the Seeds Act. It was decided, therefore, to determine whether any one of several treatments employed had an influence upon seed germination.

METHODS

The experiments were conducted from 1945 to 1949, with yield data being secured in 1946, 1947 and 1949. Because of differences in seasonal conditions, it was found impractical to sow according to predetermined dates, and, after the first year, the two field sowings were made at intervals of approximately one month. In 1946 the soil was so dry during June and July that the transplanting scheduled for the summer was not undertaken until September.

Each treatment was replicated twelve times because of lack of soil uniformity. There were differences in the size of areas available for the tests, and as a consequence individual net plot sizes varied from two 26-foot rows, 27 inches apart, in 1945–46, to two 38-foot rows, 36 inches apart, in 1948–49. It was felt, however, that this range did not affect comparisons between treatments.

Thinning was done in July when the plants were 3 to 4 inches in height, with the aid of knives fashioned from 8-inch flat mill files. At this stage the thinnings were removed without serious damage to the remaining

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plants. Transplanting was done using dibbers to make the holes for the transplants. No water was given but in all cases the plants became readily established.

In October the seed heads were cut off with approximately 2 inches of stem, using a sharp knife or hand pruner, and dried on trays in a greenhouse, with the temperature held as closely as possible to 80° F. Forced ventilation provided by fans was found necessary to permit adequate exchange of air and occasional stirring of the seed heads to promote uniform drying. Drying took approximately three weeks, after which the heads were immediately threshed and the seed cleaned by running it once over a 2-B Clipper cleaner. Plot yields were then recorded. A sample was taken from every plot, and those comprising each treatment bulked and thoroughly mixed. A single sample, representative of each treatment was then taken and forwarded to the Plant Products Division, Dominion Department of Agriculture, Vancouver, B.C., where germination tests were made.

The variety used was Musselburgh. In no case was any protection given to overwintering plants and no winter injury occurred.

RESULTS

Data on seed yield, head diameter and per cent germination were secured in 1946, 1947 and 1949 and are summarized in Table 1. While the yields were slightly high (since the seed was rough-cleaned only), the germination figures indicate that it was of a fairly good grade. Heaviest seed yields were obtained from 2-inch spacings. In all cases this treatment outyielded the 6-inch spacing sown at the same time. While the latter produced sturdier plants and larger seed heads, there was no adverse effect upon seed germination in closely spaced plots. There was a trend towards heavier yields from the earlier sowings, but in only one case—the May 6 compared to June 1 close-spacing in 1946–47—was the difference significant.

In comparing transplanting with the seed-to-seed method, no advantage was found for the former when both treatments were sown at the same time and the same spacing used. Fall transplanting was found to be much inferior, with seed yields ranging from 15.9 to 39.5 per cent of those from close-spaced field sowings.

Measurements made at harvest revealed a consistent increase in seed-head diameter of plants spaced 6 inches apart over those 2 inches apart. An exception was in the fall-transplanted plots which, though spaced at 6 inches, in nearly all cases produced the smallest seed heads.

Germination of seed from all treatments was quite satisfactory and well above the minimum of 65 per cent required under the Seeds Act for leek.

DISCUSSION

These experiments have shown that, when field sowings were made in early spring, and the plants allowed to mature where sown, high seed-yields resulted. A typical field grown by this method is shown in Figure 1. When the seedlings were permitted to remain close-spaced, the yields were greater than when thinned to 6 inches. Arguments advanced against the early field-sowing method are that weeding costs are excessive, and that it is better to raise the seedlings in a nursery-bed, transplanting to the field

TABLE 1.—YIELD* AND VIABILITY OF LEEK SEED AS AFFECTED BY TRANSPLANTING AND THINNING TREATMENTS

Year	Net Plot Size	Treatment	Seed Yield		Seed-head diameter	Germination
			Per plot	Per ac.		
			lb.	lb.	in.	per cent
1945–46	Two 26-foot rows, spaced 27 inches apart (1/372.3 acre)	A. Sown April 22, thinned to 2 inches	5.5	2048	3.3	70
		B. " " thinned to 6 inches	2.9	1080	4.0	74
		C. " May 1, transpl. 6" apart Oct. 22	1.3	484	3.4	76
		D. " " thinned to 2 inches	5.2	1936	3.3	70
		E. " " thinned to 6 inches	3.8	1414	3.9	79
		F. " June 1, transpl. 6 "apart Oct. 22	0.9	335	2.8	74
		L.S.D. (P—.05)	0.9	335		
1946–47	Two 37-foot rows spaced 27 inches apart (1/261.6 acre)	A. Sown May 6, thinned to 2 inches	7.4	1936	4.1	82
		B. " " thinned to 6 inches	5.1	1334	4.3	80
		C. " " transpl. 6" apart July 13	4.7	1230	4.2	84
		D. " June 1, thinned to 2 inches	6.6	1727	3.9	77
		E. " " thinned to 6 inches	4.9	1282	4.4	80
		F. " " transpl. 6" apart Sept.17	1.7	445	3.7	79
		L.S.D. (P—.05)	0.5	131		
1948–49	Two 38-foot rows, spaced 36 inches apart (1/191.0 acre)	A. Sown April 15, thinned to 2 inches	6.4	1222	3.5	73
		B. " " thinned to 6 inches	4.9	936	3.9	83
		C. " April 6, transpl. 6" apart July 7	4.7	898	3.9	73
		D. " May 15, thinned to 2 inches	5.9	1127	3.4	70
		E. " " thinned to 6 inches	4.7	898	3.9	67
		F. " June 1, transpl. 6" apart Sept. 20	2.5	478	3.3	67
		L.S.D. (P—.05)	0.6	115		

* Averages of 12 plots in 1945–46 and 1946–47; 11 plots in 1948–49.

FIGURE 1. A leek seed plot in full bloom in mid-August. This plot is from an early-spring field-sowing and has been allowed to stand with little thinning. Plots like this have given very satisfactory seed yields of upwards of 1000 pounds per acre.

FIGURE 2. View taken at the end of May showing inferior growth of fall transplanted (middle two rows) as compared to spring field-sown leek plants (either side).

FIGURE 3. Another view of Musselburgh leek seed plots taken in mid-September, just before harvest. Plants in the two centre rows were transplanted in the fall and have become overrrun with weeds, whereas the vigorous spring-sown plots on either side are comparatively weed-free.

in July. In the meantime, the fields can be kept cultivated and the labour costs for weeding after transplanting reduced to a minimum. While this is no doubt true, particularly in weedy fields, the results of the experiments indicate strongly that, where weeds can be kept under control without excessively high labour costs, the greatest returns will be secured from early field-sowing with close-spaced plants. In this connection, herbicides have been effective in reducing labour costs for weeding this crop and their use will undoubtedly make this method more attractive to the grower.

The practice of delaying transplanting until the fall, in order to utilize the field for an early maturing crop, has little to recommend it. As seen in Figure 2, plants grown in this way lacked vigour and yields were low. Another disadvantage was that the plants offered little competition to weeds, and, as shown in Figure 3, the plots tended to be weedier than those which were field-sown in the spring.

SUMMARY

1. Close spacing (2 inches) of field-sown leek seedlings, although producing smaller individual seed-heads, gave higher seed yields than wider spacing (6 inches).

2. Early field-sowings tended to yield more heavily than those made later, although sowings made by May 15 were not significantly less productive than those made one month earlier.

3. Similar yields were obtained from plots transplanted 6 inches apart in July and from spring field-sowings thinned to 6 inches.

4. Fall transplantings from June 1st nursery sowings resulted in inferior yields.

5. No marked differences were found in seed germination between treatments.

ACKNOWLEDGMENTS

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FRUITY FLAVOUR IN CHEDDAR CHEESE[1]

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Grading records of the Dairy Products Division, Marketing Service, show each year a small percentage of Cheddar Cheese in second and third grade because of flavour defects characterized as slightly fruity, fruity and very fruity. While it is not possible to estimate accurately the amount of money lost through this defect, it would obviously amount to many thousands of dollars annually. As the causative agents of this defect have never been thoroughly understood in terms of remedial measures, studies were undertaken with the object of obtaining more definite information on this particular flavour defect in Cheddar cheese.

Harding *et al.* (3) investigated a class of flavour defects designated as "sweet" and concluded that they were due to yeasts. Cheese of normal flavour were found to contain very few yeasts, whereas cheese showing the sweet flavour contained large numbers, at least during the first stages of ripening. The addition of cultures of certain yeasts to milk repeatedly caused off flavours in the cheese, some of which were included under the general term of "sweet" flavour.

Harrison (4) found yeasts quite common in Canadian Cheddar cheese and frequently in large numbers. He reported that yeasts were able to increase in cheese, and that a yeast count of 10,000 per gram in a two-day old cheese increased to 1,500,000 in 23 days, and then decreased to 500,000 in 45 days. Yeasts were also found in Cheddar cheese of all ages. From a 403-day old Cheddar cheese, 50 yeasts per gram were obtained. The diverse habits of yeasts were also mentioned, such as their ability to grow in acid whey and produce bad flavours and mottles in coloured Cheddar cheese when in association with starter bacteria.

Edwards (2) in 1913 isolated a number of yeasts from fruity or sweet flavoured Cheddar cheese produced in factories in Western Ontario. Experimental cheese made with various cultures of these yeasts were all bad in flavour. Prevalent flavour defects were fruity, bitter, yeasty, dirty and rancid.

To obtain information regarding the number and kinds of yeasts present, Harrison (5) examined over 100 samples of Canadian Cheddar cheese collected from Ontario and Quebec factories. From these a large number of yeasts covering a wide range of genera and species were isolated. Studies showed that when certain types were grown in milk the majority of the cultures were called yeasty, but in addition such odours as not clean, bitter, yeasty, fruity and very unclean occurred with considerable frequency. It was also shown that the flavour was often completely changed but in no case improved when yeasts were grown in milk cultures in association with lactic acid bacteria. Esters were also identified from some of the cultures. The detection of strawberry, pineapple and pear flavours suggested the presence of ethyl butyrate, ethyl acetate, ethyl

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formate and other esters in sufficient quantity to produce odours detectable as fruity through the agency of yeasts. While it was inferred by Harrison (5) that yeasts could produce similar defects in Cheddar cheese as those produced in milk cultures, this was not confirmed by making experimental vats of Cheddar cheese.

In judging cheese quality in the United States, Downs (1) states that fruity flavours are frequently referred to as sweet—suggestive of artificial pineapple.

Van Slyke and Price (8) believe the cause of fruity flavours to be due to the growth of certain yeasts or bacteria which may contaminate the milk through the use of dirty milk cans and exposure to foul air.

Hood and White (6) reported yeast counts varying from 10,000 to 3,240,000 per gram from a number of September Cheddar cheese graded as fruity. An examination of the milk supply of 39 individual patrons at one troubled factory showed yeast counts ranging from 0 to 19,000 per ml. of milk. While these workers also found, as shown in Table 1, as high yeast counts in first grade Cheddar cheese as in Cheddar cheese which had been graded as fruity, no studies were reported on types of yeasts associated with normal and fruity flavoured Cheddar cheese.

Table 1 shows the yeast count of 10 first grade and 10 second grade fruity Cheddar cheese.

TABLE 1.—YEAST COUNTS OF COMMERCIAL CHEDDAR CHEESE

No. of Sample	Yeasts per gram of First Grade Cheese	No. of Sample	Yeasts per gram of Second Grade Cheese
1	1,730,000	1	700,000 Fruity
2	1,240,000	2	300,000
3	1,260,000	3	800,000 "
4	420,000	4	100,000 "
5	22,880,000	5	1,400,000 "
6	2,130,000	6	2,000,000 "
7	1,500,000	7	4,000,000 "
8	970,000	8	2,500,000 "
9	4,640,000	9	5,500,000 "
10	478,000	10	3,000,000 "

During studies on bacteriophage in relation to Cheddar cheesemaking, Johns and Katznelson (7) encountered a vat of cheese which was designated as pronounced fruity and showed a yeast count of 1,500,000 per gram. In their opinion the defect was traceable to yeasts as the control cheese made from a portion of the same milk supply contained only 11,000 yeasts per gram and was of first grade quality.

OBJECT OF INVESTIGATION

Working on the assumption that certain types of yeasts and bacteria are responsible for fruity flavour in Cheddar cheese, a series of experimental vats of cheese were made during 1951 and 1952 from milk inoculated with selected cultures of yeasts and bacteria in order to determine their relationship to the defect.

EXPERIMENTAL

To study the effect of yeasts on cheese flavour 31 yeast cultures (Y-1 to Y-31) were isolated from degraded fruity cheese which showed yeast counts ranging from 700,000 to 5,500,000 per gram. When grown in skim milk with and without starter organisms only three of the cultures, namely, Y-16, Y-24 and Y-31, produced the characteristic fruity flavour associated with defective cheese.

As a ropy milk organism was found during earlier studies on rancid flavour in Cheddar cheese to produce typical fruity flavour, cultures of ropy milk bacteria, namely, *Alcaligenes viscosus*, *Escherichia coli* var. *neapolitana* and a *Micrococcus* species were selected to study their effect on cheese flavour.

The above cultures of yeasts and ropy milk bacteria were used individually to inoculate milk prior to making experimental vats of cheese as detailed below.

For experimental purposes 240 pounds of Experimental Farm herd milk was used for each vat of cheese. The milk was treated as follows: It was cooled to 42°F. and held at this temperature for 7 hours when it was heated to 70°F. For yeast studies vats of milk were inoculated with cultures of Y-16, Y-24 and Y-31 and allowed to incubate overnight at 70°F. to give yeast counts in the milk at setting time ranging from 2,100 to 5,500,000 per ml. The milk was then made into cheese. For bacteriological studies vats of milk were inoculated, using *Escherichia coli* var. *neapolitana*, a *Micrococcus* and two strains of *Alcaligenes viscosus*, and allowed to incubate for 48 hours at 70°F. to give bacterial counts in the milk at setting time ranging from 1,000,000 to 8,000,000 per ml. The milk was then made into cheese.

Each vat of cheese was made on a small manufacturing scale according to accepted methods for the manufacture of Canadian Cheddar cheese. Two and a half to three per cent of starter was used. The curd was pressed into cheese weighing 10 to 12 pounds. When removed from the hoops the cheese were held at 60°F. until the first grading, then placed in a 46°F. storage. They were returned to the higher temperature room for one day in order to attain a proper temperature for a second grading. The cheese were graded by experienced members of the Dairy Products Grading Staff.

ANALYSIS OF RESULTS

In Table 2 are listed 19 vats of experimental cheese which show the effect of certain types of yeast on the flavour score of the cheese. Column 2 of this table shows that three different types of yeasts were used, namely, Y-16 in vats 1 to 11, Y-24 in vats 12 to 15, and Y-31 in vats 16 to 19. In column 3 the yeast count per ml. of the milk at setting time is recorded. Columns 4, 5 and 6 give the flavour score of the cheese and flavour comments at time of the original scoring and after various periods of storage. It will be noted that in vats 1 to 5, where the milk at setting time contained high numbers of yeasts, all vats were definitely fruity at the first grading. In vats 6 to 11, where the same type of yeast was used but the milk at setting time contained a much lower number of yeasts, five vats out of six were graded as unclean. However, at the second period of grading all of the

TABLE 2.—THE EFFECT OF YEASTS ON THE FLAVOUR SCORE OF CHEDDAR CHEESE

Vat No.	Yeast Culture	Yeasts per ml. at Setting Time	1st Grading			2nd Grading			3rd Grading		
			Age of Cheese	Flavour Score	Remarks	Age of Cheese	Flavour Score	Remarks	Age of Cheese	Flavour Score	Remarks
			Days			Days			Days		
1	Y-16	5,500,000	28	37.0	Fruity	42	36.0	Fruity	231	37.0	Fruity
2	"	2,700,000	26	37.0	"	40	37.0	Fruity and Unclean	229	37.5	"
3	"	2,500,000	20	37.0	"	34	37.0	Fruity	234	37.0	"
4	"	4,000,000	14	37.0	"	28	37.0	"	218	37.5	"
5	"	1,300,000	12	37.0	"	26	37.0+	"	—	—	—
6	"	2,100	178	28.5	Sl. Fruity	214	37.0	"	325	37.0	Fruity
7	"	210,000	32	38.5	Sl. Unclean	53	37.0	"	144	37.5	"
8	"	—	25	38.0	"	46	38.0	Sl. Fruity	137	37.0	Sl. Rancid and Fruity
9	"	340,000	18	38.0	"	39	38.0	"	130	37.0	Fruity
10	"	330,000	13	37.0	Unclean	34	37.5	Fruity	125	38.0	Sl. Fruity
11	"	6,400	11	38.0	Sl. Unclean	32	38.0	Sl. Fruity	123	38.5	Sl. Fruity
12	Y-24	330,000	27	37.0	Fruity	72	37.0	Fruity	118	36.0	Fruity
13	"	97,000	25	37.0	"	60	37.5	"	116	37.5	"
14	"	5,200	20	38.5	Sl. Fruity	55	38.5	Sl. Fruity	111	37.5	"
15	"	97,000	13	37.0	Fruity	48	37.0	Fruity	104	36.5	"
16	Y-31	13,000	28	38.5	Sl. Fruity	69	39.0−	Sl. Unclean	83	39.0−	Sl. Unclean
17	"	84,000	26	37.5	Fruity	60	37.0	Fruity	81	37.5	Fruity
18	"	2,400	25	38.0+	Sl. Unclean	46	38.0	Sl. Fruity			
19	"	52,000	20	37.5	Fruity	39	36.0	Fruity			

TABLE 3.—THE EFFECT OF ROPY MILK BACTERIA ON THE FLAVOUR SCORE OF CHEDDAR CHEESE

Vat No.	Bacterial Culture	Bacteria per ml. at Setting Time	1st Grading			2nd Grading		
			Age of Cheese	Flavour Score	Remarks	Age of Cheese	Flavour Score	Remarks
						Days		
1	*Alcaligenes viscosus* Strain I	3,000,000	48	38.0	Sl. Unclean	70	37.5	Unclean
2	"	5,000,000	41	38.0	"	63	37.0	"
3	"	5,000,000	40	38.5	"	62	38.0	Sl. Unclean
4	Strain II	2,000,000	32	38.5	"	54	38.0	"
5	"	1,800,000	31	38.5	"	53	38.0	"
6	*E. coli*	8,000,000	26	38.0	"	48	36.5	Fruity
7	"	5,400,000	27	38.5	"	49	38.0+	Sl. Unclean
8	"	5,400,000	18	38.5+	"	40	38.0	"
9	*Micrococcus*	1,100,000	20	39.0	—	42	—	—
10	"	—	22	39.0	—	44	—	—

vats showed some degree of fruity flavour and were degraded for this defect. Vats 12 to 15, using Y-24 with yeast counts in the milk at setting time similar to vats 6 to 11, showed slightly fruity and fruity flavours at the initial grading. Vats 16 to 19 using Y-31 showed a similar picture at the first grading period.

The effect of some types of ropy milk bacteria on the flavour score of cheese made from 10 experimental vats is shown in Table 3. Column 2 of this table shows that four different cultures of bacteria were used, namely, strain one of *Alcaligenes viscosus* in vats 1 to 3, strain two in vats 4 and 5, *Escherichia coli* var. *neapolitana* in vats 6 to 8, and a *Micrococcus* species in vats 9 and 10. In column 3 the bacterial counts per ml. of the milk at setting time are recorded. Columns 4, 5 and 6 give the flavour score of the cheese and flavour comments at time of the original scoring and after various periods of storage. It will be noted that all of the vats were of second-grade quality at the first grading period because of slightly unclean flavour defects. At the second period of grading, vats 1 to 8 were further lowered in score owing to the presence of more pronounced flavour defects. While only one vat, namely vat 6, was found to be fruity, the flavour was typical of that found in commercial grading.

It is evident from the scores of the cheese listed in Table 2 that the extent to which the cheese were degraded for unclean, slightly fruity and fruity flavours was directly related to the number and type of yeasts in the milk supply at the time of setting.

It would also appear from the flavour scores of the cheese listed in Table 3 that, while the flavour quality of the cheese was lowered in grade because of the presence of ropy milk bacteria in the milk at setting time, fruity flavours were much less common than in the vats containing yeasts and were more difficult to reproduce experimentally than where approximately the same number of yeasts were used at setting time.

It is, nevertheless, apparent from Table 3 that ropy milk bacteria were harmful as they initiated undesirable flavours and lowered the grades in all vats of experimental cheese at the first period of grading. In addition to this, one vat of cheese was found to be typically fruity at the second grading period.

PRACTICAL IMPLICATIONS

So far as is known, no simple, rapid factory test is available for the detection of yeasts and ropy milk bacteria in cheese factory milk supplies. While the Methylene Blue Test is extensively used as a simple routine for checking the bacteriological quality or freshness of the milk, this test does not, however, detect specific contamination such as yeasts and certain fault-producing organisms. Until a practical test can be devised, the only recommendations that can be made that will assist the cheesemaker in overcoming fruity flavours in Cheddar cheese are to reject all doubtful milk supplies and encourage the production of milk of higher sanitary quality.

SUMMARY AND CONCLUSIONS

1. The effect of certain types of yeasts and ropy milk bacteria on the flavour scores of Cheddar cheese has been studied experimentally.

2. An analysis of the results of 19 experimental vats of Cheddar cheese made from milk inoculated with different types of yeasts shows consistently low flavour scores because of the presence of unclean, slightly fruity and fruity flavours..

3. An analysis of the results of 10 experimental vats of Cheddar cheese made from milk inoculated with ropy milk bacteria shows consistently low flavour scores because of the presence of slightly unclean, unclean and fruity flavours.

4. The extent to which the flavour defects developed was found to be closely related to the number of organisms present in the milk at setting time.

5. It has been demonstrated that such flavour defects as unclean, slightly fruity and fruity can be reproduced experimentally in Cheddar cheese by the addition of yeasts and ropy milk bacteria to the cheese milk. While the extent of contamination with yeasts and ropy milk bacteria is not known in cheese factory milk supplies, their presence in large numbers suggests that these organisms are a cause of the sporadic or scattered occurrence of unclean and fruity flavours in commercial Cheddar cheese.

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CLAM SHELLS, LIMESTONE AND OYSTER SHELLS, AS SOURCES OF CALCIUM IN THE RATIONS OF LAYING HENS[1]

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INTRODUCTION

The calcium and grit requirements of the laying hen are complicated by the necessity of supplying calcium for egg-shell formation to meet different rates of egg production. Adequate intake of calcium to satisfy the individual requirements of laying hens is assured by free choice feeding of a calcium supplement. Oyster shell is generally regarded as the best source of supplementary calcium, but there is ample evidence (2) that any good soluble calcium compound will serve as a source of calcium for the laying hen. This conclusion has been substantiated in a later review by Schaible (12).

However, work along this important line of research has continued. Scholes (13) found coquina shells a satisfactory source of calcium for the laying hen. Gutteridge and Pratt (4) found that egg-shells would serve as a source of calcium for laying hens. Heuser and Norris (5) conclude that limestone included in the mash is inferior to oyster shell and calcite with respect to egg production and egg-shell strength. Gutowska and Parkhurst (3) found that 8 per cent of calcite in the diet impaired feed efficiency and egg production. In a study of the sources of calcium for laying hens during hot weather, Heywang (7) found calcium sulphate was slightly inferior to the calcium carbonate of limestone, but several other compounds studied were satisfactory sources of calcium. The form in which calcite is fed seems to have little effect on the availability of the calcium for egg production and egg shell formation(8), (11). It would appear that the conclusions drawn by Branion are still valid.

Theoretically, the function of grit is to aid in the grinding of food in the gizzard, but actually its function is unknown. Branion (2) concludes that it is a wise practice to use insoluble grit, although it does not appear to be essential, and Schaible (12) concurs with this conclusion. Subsequent work has confirmed the opinion of these authors (6), (1), (10), (14).

EXPERIMENTAL

The work reported herein was designed to study the relative effectiveness of clam shells, limestone and oyster shells as sources of calcium for laying hens, and to study the effect of feeding insoluble grit in addition to oyster shells. The oyster shells, clam shells and ground limestone were purchased from local dealers and were all of good quality. The insoluble grit was a commercial silicate grit. The chemical analysis of the calcium supplements and the insoluble grit are shown in Table 1. The small amount of insoluble material in the limestone is largely ferric oxide and as such is not considered to be detrimental to the availability of the calcium in this material. The hardness of these materials varies considerably, as shown in the table. Hardness was measured by grinding particles which

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TABLE 1.—CHEMICAL ANALYSIS, HARDNESS AND SOLUBILITY OF OYSTER SHELLS, CLAM SHELLS, LIMESTONE AND INSOLUBLE GRIT

	Per cent				Hardness	
	Calcium	Calcium Carbonate	Moisture	Insoluble Material	% Passing 100 mesh(*) Screen	% Soluble in HCl
Oyster shells.....	38.2	95.4	0.25	Nil	4.12	5.58
Clam shells.....	38.6	96.4	0.33	Nil	3.95	5.20
Limestone.....	39.8	99.4	0.09	0.6	10.07	6.08
Insoluble grit.....	Nil	Nil	0.01	98.9	.....	.....

* Particles which would pass a 5-mesh screen, but were retained on a 10-mesh screen were ground in a ball mill for 2 hours and the per cent of the grindings which would pass a 100-mesh screen determined.

would pass through a 5-mesh screen but were retained on a 10-mesh screen, in a ball mill for two hours, and measuring the amount of fine material resulting which would pass a 100-mesh screen. The solubility of this fine material was then tested in a solution of hydrochloric acid of pH 3. The clam shells are slightly harder and less soluble than the oyster shells or limestone. From the point of view of a grinding material or grit the clam shells would appear to be superior to oyster shells or limestone.

This work was begun in December and continued through August, a total of 252 days. There were 800 mature Barred Plymouth Rock hens under test. The birds were randomized into 40 similar pens in two laying-houses. There were 20 pens in each house, with 20 birds in each pen. The birds were selected at random from about 1,000 six-month-old pullets, after birds with obvious abnormalities were culled out. The pullets were all of the same age and raised on the same range under identical conditions.

Four treatments were imposed, with 10 pens of 20 birds on each treatment. The treatments differed in the calcium supplement fed. In each case the supplement was added to the all-mash ration and also fed ad lib. in grit hoppers in the pens. The basal ration was a coarsely ground, all mash ration of the following composition: ground oats, 30.0 per cent; ground barley, 23.5 per cent; ground wheat, 33.9 per cent; wheat bran, 2.5 per cent; white-fish meal, 5.0 per cent; Vita-gras, 2.5 per cent; bone meal, 1.5 per cent; salt, 0.5 per cent; fish oil (1,000 A, 200 D), 0.6 per cent; manganese sulphate, 0.25 pounds per ton; riboflavin, 0.5 grams per ton. The calcium supplement which was added to the ration was finely ground. The calcium supplement fed ad lib. was in the usual coarsely broken form supplied for laying hens. The four treatments were as follows:

(1) Basal ration plus 1½ per cent finely ground oyster shell; coarsely broken oyster shell fed ad lib.

(2) Basal ration plus 1½ per cent finely ground clam shell; coarsely broken clam shells fed ad lib.

(3) Basal ration plus 1½ per cent finely ground limestone; coarsely broken limestone fed ad lib.

(4) Basal ration plus 1½ per cent finely ground oyster shell; coarsely broken oyster shell and insoluble grit fed ad lib.

TABLE 2.—ANALYSIS OF VARIANCE OF PEN AVERAGES

Source of Variation	Degrees of Freedom	Average Egg Production		Per Cent Egg Production		Feed Consumption		No. of Eggs per 100 lb. of Feed	
		Variance	F. Value	Variance	F. Value	Variance	F. Value	Variance	F. Value
Total	39	186.035	—	19.3502	—	1.5822	—	234,463	—
Between houses	1	198.025	1.05	5.2563	(*)	12.8369	9.79	284,622	1.49
Among treatments	3	106.892	(*)	1.1569	(*)	0.8116	(*)	21.611	(*)
Houses X treatments	3	15.358	(*)	6.0062	(*)	0.6671	(*)	76.260	(*)
Between replicates within houses	8	272.637	1.45	30.2589	1.49	1.6232	1.24	498.975	2.62
Error	24	187.896	—	20.2430		1.3103	—	190.584	—

* Variance less than error variance.

Source of Variation	Ca. Supp. Consumption		Av. Final Body Weight		Per cent Mortality		F. Value Necessary for Significance	
	Variance	F. Value	Variance	F. Value	Variance	F. Value	P = 0.05	P = 0.01
Total	.009919	—	.036754	—	21.999	—	—	—
Between houses	.000903	(*)	.184960	5.87	27.390	1.08	4.26	7.82
Among treatments	.053305	11.20	.002273	(*)	32.708	1.28	3.01	4.72
Houses X treatments	.020309	4.27	.015700	(*)	20.419	(*)	3.01	4.72
Between replicates within houses	.006345	1.33	.054886	1.74	7.649	(*)	2.36	3.36
Error	.004758	—	.031476	—	25.415	—	—	—

The randomization of the treatments throughout the pens was restricted so as to permit the measurement of house differences and the position of pen in house differences without prejudice to treatments. The randomization was such as to be amenable to variance analysis according to the design shown in Table 2.

Gross egg production was recorded daily for each pen. In addition individual egg records were maintained for all birds in four pens on each treatment for the first 140 days. A representative sample of eggs from these pens was tested for shell strength by the specific gravity method of Olsson, as outlined by Munro (9).

An accurate record of feed, calcium supplement and grit consumption was maintained for all pens by weighing back the unconsumed portion remaining in the hoppers at regular intervals. Individual bird weights were recorded three times during the experiment.

The pen averages for all the criteria shown in Table 3 were analysed by analysis of variance as shown in Table 2, and the individual values for specific gravity were analysed as shown in Table 4.

RESULTS AND DISCUSSION

The response of laying hens to oyster shells, clam shells, limestone and oyster shells plus insoluble grit as calcium supplements were the same for all of the criteria measured except calcium consumption and egg-shell quality (Tables 3 and 4). In Table 3 the data shown were collected on a pen basis. In Table 4 the data shown were obtained from individual bird records.

There are no significant differences in feed consumption except between houses which is attributed to difference in personnel in the two houses; hence it must be concluded that the different sources of calcium studied had no effect on feed consumption or feed efficiency for laying hens and that insoluble grit fed in addition to oyster shells was of no benefit in this regard.

With the exception of the limestone the consumption of the calcium supplements was about the same, and the addition of insoluble grit had no

TABLE 3.—EFFECT OF DIFFERENT CALCIUM SUPPLEMENTS AND GRIT ON LAYING HENS

Criterion of Comparison Means of 10 Pens	Treatment			
	Oyster Shell	Clam Shell	Lime-stone	Oyster Shell and Grit
Average egg production per bird. Hen housed basis	147	146	153	147
Per cent egg production	63	62	63	63
Pounds of feed per 100 birds per day	30.6	30.0	30.0	30.3
Number of eggs per 100 pounds feed	206	207	209	207
Pounds Ca. Supp. per 100 birds per day	0.82	0.80	0.66	0.80
Average final body weight (pounds)	6.25	6.25	6.20	6.25
Per cent mortality	7.5	7.0	3.5	7.0

TABLE 4.—ANALYSIS OF VARIANCE OF SPECIFIC GRAVITY OF EGGS FROM INDIVIDUAL HENS

	Treatment			
	Oyster Shell	Clam Shell	Lime-stone	Oyster Shell and Grit
Number of birds tested	76	76	76	78
Av. specific gravity of eggs	1.0729	1.0739	1.0725	1.0748
Standard deviation	±0.0052	±0.0059	±0.0045	±1.0045

Source of variation	Degrees of Freedom	Variance*	V. Value	
			Observed	Necessary P=0.05
Total	302	2731.38		
Among treatments	3	8020.48	3.00	2.64
Among blocks	3	6388.86	2.39	2.64
Blocks X Treatments	9	1705.50	0.64	1.92
Within sub classes	287	2670.03		

* Figures are from coded values for specific gravity. (Specific gravity x 10,000)

effect on the amount of oyster shell consumed. The amount of insoluble grit consumed was quite small in relation to the amount of calcium carrier, the actual amount of grit being 0.0026 pounds per bird per day compared with a total oyster shell intake of 0.0125 pounds per bird per day. The reason for the low consumption of limestone is not clear but may be due to the softer limestone being more readily available to the bird. Egg production was not adversely affected by the lower consumption of limestone.

Egg-shell strength or thickness as determined by the specific gravity method shows that the birds receiving both oyster shells and grit maintained stronger shells than the birds receiving oyster shells alone, or limestone (Table 4). When these values are compared significant differences are noted between the oyster shell plus grit group, and the groups fed oyster shells alone and limestone. Clam shells appear to be just about as efficient as oyster shells and grit in maintaining good egg-shell quality. The lower consumption of limestone might probably account for the poor shell quality from this group, but the poor quality shells from the birds fed oyster shells alone cannot be accounted for on this basis. Table 1 shows the hardness of the calcium carriers, and the solubility of the same carriers when treated with a solution of hydrochloric acid of pH 3, for five hours on a steam bath, the softer materials being the more soluble. The fact that clam shells, or insoluble grit in addition to oyster shells, improves the quality of the egg-shells indicates that the hard material has a favourable effect on egg-shell formation, but the reason for this is obscure. Heuser and Norris (5) report similar beneficial effects on egg-shell breaking strength from feeding a hard granite grit in addition to oyster shells or calcite grit.

They rate oyster shells superior to calcite grit and calcite grit superior to ground limestone. This would seem to indicate the need for a hard coarse calcium supplement or a hard grit in addition to the calcium supplement for maintenance of superior egg-shell quality.

SUMMARY

1. Oyster shells, clam shells, ground limestone, and oyster shells plus insoluble grit were compared as sources of calcium for the laying hen.

2. There was no difference in egg production, feed consumption or maintenance of body weight between the hens fed the different sources of calcium or upon the addition of insoluble grit with oyster shell.

3. Birds fed clam shells, or oyster shells plus insoluble grit, produced egg-shells of superior quality to those fed oyster shells or limestone alone.

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A STUDY OF DESICCATION IN RELATION TO WINTER INJURY[1]

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INTRODUCTION

Moisture content of plants has frequently been associated with their cold resistance. This is not surprising, since the phenomenon of killing by cold appears to revolve around the extent of dehydration of the cell content on the one hand, and the amount of water frozen either extra- or intra-cellularly on the other. According to Maximov (4) the basis of resistance of a plant to cold lies in the ability of its protoplasm to withstand disintegration following desiccation. Wiegand (8) from his studies on behaviour of buds and twigs of trees in winter also concluded that "every cell has its critical point beyond which the withdrawal of water, whether by evaporation or other means, will cause death". He also attributed this to the fact that the delicate structure of the protoplasm cannot sustain itself when too many molecules of water are removed from it. The importance of studying factors affecting desiccation of twigs during winter on the Canadian prairies in relation to winter injury is thus self-evident.

EXPERIMENTAL PROCEDURE

Exploration studies were conducted during the winters of 1950 and 1951 to investigate the relative effects of temperatures above and below freezing on the extent of desiccation of one-year-old mature twigs of *Caragana arborescens* (Lam.), *Fraxinus pennsylvanica* var. *lanceolata* (Sarg.), *Ulmus americana* (L.), *Acer Negundo* (L.) and *Populus deltoides* (Marsh.). Trees of these species were grown since 1943 in belts 4 × 4 ft., 8 × 8 ft., 16 × 16 ft., and 4 × 4 ft. with mulch. As shown by Macoun (2) the terminal growth appears to be most frequently injured under prairie conditions, and this injury is indicative of inherent tenderness of the variety or of immaturity of tissues.

The winter temperatures for the periods the study was in progress in 1950 and 1951 are shown in Table 1.

The data in Table I indicate that a relatively colder period of 60 to 62 days and a milder period of 45 to 43 days prevailed during the 105 days of outdoor exposure of twigs in 1950 and 1951. These colder periods were characterized by relatively few days with temperatures above freezing, especially above 41° F., which, according to Meyer and Anderson (5), could be considered high enough to stimulate metabolic activity and to deharden plant tissues. The mean temperatures for these periods in 1950 and 1951 were −2.4 and 0.9° F. respectively with extreme minima ranging from −42 to −36° F. and an extreme maximum of 42° F. During the milder periods in 1950 and 1951 there were numerous days with temperatures above freezing. The mean temperatures of the milder periods were about 30 to 36° F. higher as compared to the more severe period. The data also indicate that for the period of study relatively higher temperatures

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TABLE 1.—RECORDED TEMPERATURES BETWEEN DATES OF COLLECTION OF TWIGS DURING THE PERIODS OF STUDY IN WINTERS OF 1950 AND 1951

Temperatures	Periods of study			
	1950		1951	
	Jan. 12 [1] to Mar. 13 [2] (60 days)	Mar. 14 to April 26 [3] (45 days)	Jan. 25 [1] to Mar. 28 [2] (62 days)	Mar. 29 to May 10 [3] (43 days)
Number of days with temperatures above 32° F.	3	33	6	34
Number of days with temperatures above 41° F.	1	12	1	22
Mean temperature for the specified periods in °F.	− 2.4	27.2	0.9	37.6
Extreme minimum temperature in °F.	−42.0	−16.0	−36.0	5.0
Extreme maximum temperature in °F.	42.0	57.0	42.0	73.0

[1] Date of beginning of study and first collection of twigs.
[2] Date of second collection of twigs from desiccation rack and trees in field.
[3] Date study terminated and third collection of twigs from desiccation rack and trees in field.

prevailed during the milder period of 1951 as compared to 1950. It is apparent that by delaying the study to January 25 in 1951 as compared to January 12 in 1950 it was possible to gain additional information on effects of mild spells, commonly experienced in chinook regions on the prairies, on certain physiological behaviour of woody plants. The delay, however, did not greatly affect the water content of mature twigs taken from several species of trees at the beginning of 1950 and 1951 studies. This is shown by the data given in Table 2.

To determine the extent of desiccation of the windbreak species during the periods of exposure indicated in Table 1, four bundles of 8 to 10 twigs each were prepared from a thoroughly mixed supply of twigs. These were taken at breast height and around the whole perimeter of the selected tree representing each cultural practice. In order to decrease the extent of variability due to soil heterogeneity and other causes, the twig samples were taken from the same trees throughout this study. The basal ends were sealed with grafting wax to prevent moisture losses from cut surfaces. The twigs were exposed on outdoor drying racks. The differences in weight of these bundles before, during, and after outdoor exposure, were considered as mainly due to water losses.

The sample of twigs from each selected tree also was tested for water content by drying twigs at a constant temperature of 101.0° C. for 48 hours in a thermostatically controlled oven. As many twigs as would fill four desiccation boxes were used. Each box held about two or three twigs, cut into sections of suitable length. The total water content was calculated as per cent of fresh weight. At each weighing the water content of twigs from the desiccation rack was compared with twigs taken in a similar manner from the same trees in field that were used throughout this study. This supplementary study was introduced to gain fundamental knowledge pertaining to plant behaviour under chinook conditions.

TABLE 2.—WATER CONTENT, EXPRESSED AS PER CENT OF FRESH WEIGHT, OF MATURE ONE-YEAR-OLD TWIGS TAKEN FROM THE SAME TREES OF GIVEN SPECIES AT THE BEGINNING OF 1950 AND 1951 STUDIES [1]

Plants [2]	Jan. 12, 1950	Jan. 25, 1951
Caragana	45.4	46.2
Green ash	35.5	35.9
American elm	45.9	46.4
Boxelder	48.2	50.7
Cottonwood	48.7	49.4
L.S.D. at P .05	2.3	2.1

"t" value for difference between water content of twigs = 2.535
"r" " " association " " " " " = .991(**)

[1] All figures in Table are averages of four trees, each grown since 1943 in belts 4 × 4 ft., 8 × 8 ft., 16 × 16 ft., and 4 × 4 ft. with mulch. Since the analysed data indicated no significant differences due to the cultural practices (9), the averages of the four trees were considered representative of each named species. Each tree was represented by four individual samples of twigs.

[2] The plants were arranged in a decreasing order of winter survival as commonly recognized by their field performance.

(**) Significant at P .01.

The extent of winter injury following two types of exposures and two periods with varying temperatures was quantitatively determined in inches. This criterion of winter injury was also used by Hildreth (2) who worked along similar lines. Every tree was represented in each treatment by a random sample of four twigs. The twigs from the desiccation rack were cut to remove the waxed basal ends and together with those collected from trees were kept in water in greenhouse for about two weeks to allow the development of changes following winter injury (2). The portion of the twig with unhealthy discoloration, lack of flexibility on manipulation, etc., was considered to be killed.

The data on water losses and extent of tip killing were analysed separately for each species by statistical methods given by Snedecor (7). The calculated values to indicate significant differences or degree of association are given with the basic data as shown in each table.

EXPERIMENTAL RESULTS

Table 3 presents the data on extent of winter desiccation of twigs of five windbreak species during periods when relatively severe and mild temperatures were encountered in this study.

The data in this table indicate that in general the water content of twigs taken from trees in field was not greatly affected during the 60-day period ending March 13, 1950, or the 62-day period ending March 28, 1951. During the same periods a certain amount of water loss was indicated by twigs exposed on the drying rack. However, with the exception of boxelder in 1950 and cottonwood in 1950 and 1951, none of the differences between the water content of twigs exposed on rack and on trees in the field has attained the value necessary for statistical significance. The higher temperatures that prevailed during the following 45 days ending April 26, 1950, and 43 days ending May 10, 1951, resulted in a significantly lower water

TABLE 3.—WATER CONTENT, EXPRESSED AS PER CENT OF FRESH WEIGHT, OF MATURE ONE-YEAR-OLD TWIGS OF FIVE WINDBREAK SPECIES FOLLOWING TWO PERIODS OF EXPOSURE, ON DESICCATION RACK, AND ON TREES IN FIELD, DURING WINTERS OF 1950 AND 1951 [1]

Plants	Type of exposure	1950				1951			
		Period ending March 13		Period ending April 26		Period ending March 28		Period ending May 10	
		Water content	Difference in W.C. [2]	Water content	Difference in W.C. [2]	Water content	Difference in W.C. [3]	Water content	Difference in W.C. [3]
Caragana	Desiccation rack	43.4	(−2.0)	35.6	(−9.8)	43.6	(−2.6)	32.5	(−13.7)
	Trees in field	44.6	(− .8)	48.5	(3.1)	45.1	(−1.1)	54.9	(8.7)
	L.S.D. at P .05	1.3		1.3		2.4		2.4	
Green ash	Desiccation rack	34.3	(−1.2)	30.7	(−4.8)	34.5	(−1.4)	29.3	(− 6.6)
	Trees in field	33.0	(−2.5)	41.8	(6.3)	34.3	(−1.6)	51.7	(15.8)
	L.S.D. at P .05	3.0		3.0		6.9		6.9	
American elm	Desiccation rack	43.3	(−2.6)	33.6	(−12.3)	43.0	(−3.4)	30.5	(−15.9)
	Trees in field	45.2	(− .7)	51.1	(5.2)	45.5	(− .9)	53.3	(6.9)
	L.S.D. at P .05	2.8		2.8		2.6		2.6	
Boxelder	Desiccation rack	45.9	(−2.3)	37.1	(−11.1)	47.6	(−3.1)	33.8	(−16.9)
	Trees in field	49.6	(1.4)	54.8	(6.6)	48.9	(−1.8)	66.8	(16.1)
	L.S.D. at P .05	1.9		1.9		5.1		5.1	
Cottonwood	Desiccation rack	45.8	(−2.9)	35.5	(−13.2)	44.1	(−5.3)	30.4	(−19.0)
	Trees in field	49.9	(1.2)	53.4	(4.7)	47.1	(−2.3)	52.7	(3.4)
	L.S.D. at P .05	1.2		1.2		1.4		1.4	

Other Statistical Values Pertaining to the Study

Degree of association (r) between the water content of twigs of plants determined on January 12, 1950, (Table 2) and their water loss determined on April 26, 1950 = .949(*)
Degree of association (r) between the water content of twigs of plants determined on January 25, 1951, (Table 2) and their water loss determined on May 10, 1951 = .959(**)
Degree of association (r) between water loss of twigs determined on April 26, 1950 and May 10, 1951 = .804
"t" value for difference between water loss of twigs of plants following outdoor exposure on drying rack at end of 1950 and 1951 studies = 5.55(**)
"t" value for difference between water gains of twigs of plants following outdoor exposure on trees in field at end of 1950 and 1951 studies = 2.34

(*) Significant at P .05; (**) Significant at P .01

[1] All figures in table are averages of four trees, each grown since 1943 in belts 4 × 4′; 8 × 8′; 16 × 16′; and 4 × 4′ with mulch. Since the analysed data indicated no significant differences due to the cultural practices, the average of the four trees were considered representative of each named species. Each tree was represented by four individual samples of twigs.

[2] Difference in water content of twigs as compared to January 12, 1950 (Table 2).

[3] Difference in water content of twigs as compared to January 25, 1951 (Table 2).

content of all twigs exposed on the rack and a higher water content of similar twigs taken from trees in the field. However, during both periods the rate of desiccation by all twigs on the rack, except ash, was in excess of the water gained by twigs in field.

The data in Table 3 also indicate that, as a result of the 105-day exposure treatment on the drying rack, the average water loss of twigs of caragana, ash, elm, boxelder and cottonwood in 1950 was 9.8, 4.8, 12.3, 11.1, and 13.2 per cent of fresh weight, respectively. The water loss of these species in 1951 was 13.7, 6.6, 15.9, 16.9, and 19.0 per cent of fresh weight, respectively. No significant association was found between the water losses of these species following the same number of days of winter exposure in 1950 and 1951. However, the differences in these losses were significant, whereas the differences in the water gains of similar twigs taken from trees in the field were not. The analysed data also revealed that in both 1950 and 1951 a significant positive association existed between the water content of twigs of the selected plants determined at the beginning of the study and the extent of their desiccation during the outdoor exposure on the rack.

The analysed data in Table 4 indicate that significantly more injury to twigs followed the same length of exposure in 1951 as compared to 1950.

TABLE 4.—EXTENT OF TIP KILLING, IN INCHES, OF MATURE ONE-YEAR-OLD TWIGS OF FIVE WINDBREAK SPECIES FOLLOWING TWO PERIODS OF EXPOSURE, ON DESICCATION RACK, AND ON TREES IN FIELD, DURING WINTERS OF 1950 AND 1951 [1]

Plants	Type of exposure	1950		1951	
		Mar. 13	April 26	Mar. 28	May 10
Caragana	Desiccation rack	.1	.9	.2	4.5
	Trees in field	.0	.0	.0	.0
	L.S.D. at P .05	—		2.3	
Green ash	Desiccation rack	.0	.0	.0	.0
	Trees in field	.0	.0	.0	.0
	L.S.D. at P .05	—		—	
American elm	Desiccation rack	.0	1.5	.9	8.1
	Trees in field	.0	.0	.0	.0
	L.S.D. at P .05	—		2.2	
Boxelder	Desiccation rack	.0	.0	.0	2.0
	Trees in field	.0	.0	.0	.0
	L.S.D. at P .05	—		1.4	
Cottonwood	Desiccation rack	.1	.0	.6	5.8
	Trees in field	.0	.0	.3	.0
	L.S.D. at P .05	—		1.5	

"t" value for difference in tip killing of the selected plants following the 105-day period of exposure in 1951 as compared to 1950 = 2.83 (*).

[1] All figures shown in table are averages of four trees each grown since 1943 in belts 4 × 4 ft., 8 × 8 ft., 16 × 16 ft., and 4 × 4 ft. with mulch. Since the analysed data indicated no significant differences due to cultural practices, the averages of the four trees were considered representative of the named species. Each tree was represented by four twigs.

* Significant at P .05.

The increased injury resulted mainly from the exposure of twigs on the drying rack during the period of March 29 to May 10, 1951. During this period all twigs, with the exception of ash, suffered significantly more killing than similar twigs taken from trees in the field or those exposed on the rack for the period of 60 days prior to March 29, 1951.

DISCUSSION

The data obtained from this exploratory investigation revealed that the water content of mature twigs of caragana, ash, elm, boxelder and cottonwood, collected at the beginning of 1950 and 1951 winter studies, has not been significantly affected by the cultural practices of growing trees in belts 4 × 4 ft., 8 × 8 ft., 16 × 16 ft., and 4 × 4 ft. with mulch (9). The mean differences between water content of these species resulting from the four cultural treatments were highly significant on January 12, 1950, and highly associated with the mean differences in the water content of mature twigs taken from the same trees on January 25, 1951. This indicates a certain constancy in woody plants with respect to the water content at maturity, which may possibly be an inherent trait.

The data indicated that in both 1950 and 1951 the water content of mature twigs exposed on a drying-rack or on trees was not greatly affected during periods with temperatures below freezing. During both winters desiccation of twigs on the rack occurred mainly during periods with temperatures above freezing. The desiccating effects of such temperatures, especially those above 41° F. commonly experienced in chinook regions on the prairies, is thus indicated. The data on water content of twigs taken from trees in the field showed that during the milder periods of the winter significantly greater increases in water content were noted in all twigs. This indicates—as also shown by Hildreth with apples (2)—that water has been conducted into twigs in excess of the losses shown by similar twigs on the drying rack. However, with the exception of ash, the amount of water conducted in excess of the losses was less than the amount lost from twigs on the drying rack. The significantly greater water loss revealed by twigs on the rack following the relatively milder periods in 1951 as compared to 1950, without the corresponding significant gains by similar twigs taken at the same time from trees in field, may be further evidence of the desiccating effects of mild spells on the prairies. The possible greater suitability of ash trees in the chinook areas is also indicated by both 1950 and 1951 data.

The positive association that was found in 1950 and 1951 between water content of mature twigs and their water losses during the period of exposure is taken to indicate that woody twigs with more moisture may lose more when exposed to certain desiccating atmospheric conditions. The possibility is not overlooked that a certain association may exist between greater maturity and less water loss in woody twigs. No evidence was found to show that a relationship existed between the extent of desiccation of the selected plants during the two winters. During each winter all plants were influenced primarily by milder atmospheric conditions as encountered in this study.

The study revealed that significantly more tip injury to the twigs on the desiccation-rack followed outdoor conditions with higher temperatures in 1951 as compared to 1950. The greater injury in 1951 was mainly the result

of more tip killing during a 43-day period with temperatures ranging from 5 to 73° F. and 22 days above 41° F. as compared to a 62-day period with temperatures ranging from −36 to 42° F. Such widely fluctuating temperatures are not uncommon during winters under chinook conditions on the prairies. This is taken to indicate that the higher winter temperatures resulted in greater injury to woody twigs than extreme low temperatures that prevailed during the period this study was in progress. It may be that other physiological changes have occurred as a result of the 21 additional days with temperatures above 41° F. such as dehardening of twigs, etc. However, since these assumed changes in twigs have not resulted in any apparent injury to tips of similar twigs taken at the same time from trees in field that have not shown any moisture loss, it is obvious that the serious injury to twigs on a drying rack resulted to a great extent from the desiccating effects during the periods with higher temperatures.

The behaviour of ash indicated an association between lower water content, i.e. greater maturity, and higher winter survival. The association is substantiated to a certain extent by the generally known satisfactory performance of this windbreak species on the prairies, and is also in accordance with findings reported by others (1, 3, 6). However, further work with an artificially-controlled freezing unit will be required for a more definite conclusion on water content of mature twigs and its relation to winter injury. No evidence was found in this study to support Macoun's (3) and Shutt's (6) beliefs that water content at maturity, and the subsequent hardiness of twigs, may be qualities that can be affected by cultural practices. This is a topic of discussion in another paper (9).

SUMMARY

Studies of an exploratory nature were conducted during the winters of 1950 and 1951, to investigate the effects of mild spells, commonly experienced in chinook regions on the prairies, on desiccation and winter injury of mature one-year-old twigs taken from trees of caragana, ash, elm, boxelder and cottonwood. Analysed data revealed the following:—

1. During both winters the water content of twigs of these species exposed on an outdoor drying rack or attached to trees was not greatly affected during periods with temperatures below freezing.

2. Desiccation of twigs on the rack occurred mainly during periods with temperatures above freezing, especially above 41° F. During these milder periods greater increases in water content were found in all twigs taken from trees in field, which suggested that water had been conducted into twigs to overcome any water loss that had occurred. However, with the exception of ash, the amount of water conducted in excess of the losses was less than the amount lost by twigs on the rack.

3. During the mild spells encountered in this study the twigs of ash as compared to other species studied lost the least moisture on the drying rack and gained the most in the field. This would indicate a possible greater suitability of this species in the chinook areas of the prairies. The mature twigs of ash had the lowest moisture content of the selected trees at the beginning of the study and did not suffer any killing on drying racks or on trees in field during the 105-day period of outdoor winter exposure. This

would indicate a possible association between greater maturity and higher winter survival as suggested by some workers. Further work with an artificially-controlled freezing unit will be required for a more definite conclusion on water content of mature twigs and its relation to winter injury.

4. The study revealed that the desiccating effects of mild spells in winter resulted in greater injury to woody twigs exposed on a drying rack than extreme low temperatures that prevailed during the period this study was in progress. The desiccation injury, however, was not apparent on twigs that remained attached to trees since these were able to restore, in part, the moisture loss indicated by twigs on the drying rack.

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EFFECTS OF THE APPLICATION OF FUNGICIDES TO WOUNDED PLANT TISSUES[1]

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INTRODUCTION

Prior studies have shown that certain fungicides when applied to cut or wounded surfaces of a wide variety of plant propagation material exerted either beneficial or harmful effects irrespective of plant species. Application of Arasan and Spergon dusts to freshly cut potato sets exerted a beneficial effect, as measured by freedom from rot, after contact with soil artificially infested with *Pythium ultimum* Trow. (1); to cypress cuttings, as measured by the number that rooted and survived (2); to artificially wounded iris and tulip bulbs, as measured by freedom from Penicillium rot, after subsequent periods of moist storage (3); and to hyacinth scales, as measured by the subsequent yield of bulblets (4). On the other hand, the same studies revealed that red copper oxide dust was injurious to potato sets, artificially wounded iris and tulip bulbs, and to hyacinth scales, as compared with the untreated, and all the fungicides tested that contained either copper or mercury exerted either neutral or injurious effects. These studies were extended owing to the practical importance of discovering why certain fungicides can be used safely in the treatment of injured tissue, regardless of the plant species concerned.

EXPERIMENTAL

The Protection of Tuber Sets against Fusarium Rot

Trials were conducted similar to the one already reported (1), except that the sets were planted in soil heavily infested with a *Fusarium* sp., a form that was usually present in partially decayed potato sets from local farms, and with *Fusarium caeruleum* (Lib.) Sacc. from potato tubers, a culture received from G. B. Sanford. The results are given in Table I.

TABLE 1.—PERCENTAGE OF ROTTED SETS THREE WEEKS AFTER PLANTING

Fungus	Dust treatments					
	Arasan	Fermate	Spergon	Copper oxide	Control	Error 5% level
Fusarium sp.	60	48	80	100	92	14
F. caeruleum	2	16	25	100	80	19

[1] Contribution No. 1174 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada.

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The Influence of Fungicides upon Moisture Loss from Cylinders of Potato Tissue

Groups of 40 potato tuber tissue cylinders, 1 × 1.5 inch, were dusted with red copper oxide, hydrated lime, sulphur, Arasan, Fermate, and Spergon, and similar groups were immersed for 2 minutes in a 1-1000 solution of mercuric chloride, and another group in a 1-75 solution of Semesan Bel. These cylinders were maintained in an atmosphere saturated with moisture for 4 days. They were then weighed, transferred to a dry atmosphere, and reweighed at intervals of 24 hours over a 4-day period. At the end of this period, the total moisture losses in descending order by treatment were as follows: copper oxide 72.0 per cent; mercuric chloride 71.8 per cent; Semesan Bel 71.2 per cent; Fermate 69.2 per cent; Spergon 67.7 per cent; Arasan 67.1 per cent; lime 66.2 per cent; sulphur 64.8 per cent; and no treatment 64.6 per cent. The error at the 5 per cent level was 1.6. The moisture losses from the cylinders treated with all the fungicides, except sulphur, were greater than from the untreated cylinders. Even hydrated lime appeared to increase slightly the water loss. However, it is noteworthy that the moisture losses were greatest from cylinders treated with copper oxide dust, mercuric chloride, and Semesan Bel solutions.

The Effect of Fungicides and Naphthalene Acetic Acid on the Rooting of Cuttings

The effect of dusting the cut bases of cuttings with undiluted Arasan, Fermate, Spergon, Ceresan, and Semesan with and without the addition of 10 p.p.m. naphthalene acetic acid on their survival after planting in sand and peat is shown by the data of Table 2.

TABLE 2.—THE NUMBER OF CUTTINGS OUT OF GROUPS OF 50 THAT ROOTED AND SURVIVED AS AFFECTED BY FUNGICIDAL DUSTS AND NAPHTHALENE ACETIC ACID

Dust	*Cupressus lawsoniana erecta viridis* Cy.	*Tamarix parviflora* D.C.	*Sequoia washington-iana* Ag.	*Ilex aquifolium* L.
Arasan	17	45	8	9
Fermate	8	37	14	8
Spergon	23	21	6	9
Ceresan	0	20	—	—
Semesan	3	29	—	—
Arasan + NAA*	12	40	1	14
Fermate + NAA	10	42	11	15
Spergon + NAA	14	15	2	17
Ceresan + NAA	0	27	—	—
Semesan + NAA	1	23	—	—
Controls				
No treatment	8	24	1	5
NAA alone	6	15	0	15
Error 5% level	4	4	4	3

* NAA Naphthalene acetic acid, 10 p.p.m.

DISCUSSION

These studies support those previously reported (1, 2, 3, 4) by showing that the application of Arasan and Spergon to wounded plant tissues exerts a favourable effect, and this effect is independent of the plant species. Applications to potato sets exerted a favourable effect, as measured by the percentage free from rot, after planting in soil artificially infected with *Fusarium caeruleum* (Lib.) Sacc. and an unidentified *Fusarium* sp. Applications of Arasan to the base of cypress, tamarisk, sequoia, and holly cuttings exerted a favourable effect, as measured by the numbers that rooted and survived. Applications of Spergon also exerted a favourable effect, except upon tamarisk cuttings. These studies also support those previously reported by showing that the application of fungicides containing copper and mercury are frequently injurious when applied to wounded plant tissue. Dusting potato sets with copper oxide increased the percentage that rotted through attack by *Fusarium* spp., and the dusting of the bases of cypress cuttings with Ceresan and Semesan reduced the number that rooted and survived. The application of Fermate to potato sets proved to be particularly favourable in preventing tuber-set rot, and this fungicide exerted a favourable influence upon the rooting of tamarisk and sequoia cuttings. However, the favourable influence of Fermate was not independent of plant species. This dust treatment failed to increase the number of cypress cuttings that rooted and survived.

These results may be explained by assuming that the natural healing of the cut surfaces of potato sets and cuttings plays a part in their survival, and that Arasan, Spergon, and Fermate interfere less with natural healing than fungicides containing copper or mercury. This assumption is supported by the study of moisture loss from potato tuber tissue cylinders. The greater moisture losses after treatments with copper oxide and mercury compounds compared with the Arasan, Spergon, and Fermate dust treatments, suggests that the former interfere more than the latter with natural healing. It is well known that the suberin layer that forms at the surface of cut potato sets checks the loss of moisture.

Since natural healing appears to play a part in the survival of cuttings, it seemed probable that the inclusion of a hormone with the dust treatment might prove beneficial. The data in Table 2 do not support such a conclusion, except in the case of holly cuttings. The inclusion of 10 p.p.m. naphthalene acetic acid with Arasan, Spergon, and Fermate applied to cypress, tamarisk, and sequoia cuttings failed to exert a beneficial effect. On the other hand, the inclusion of the hormone exerted a pronounced beneficial effect upon holly cuttings as measured by the number that rooted and survived.

SUMMARY

The application of Arasan and Spergon dusts to wounded plant tissues, potato sets, and cuttings of cypress, tamarisk, sequoia, and holly usually exerted favourable effects as measured by the protection of the potato sets against Fusarium rot and by the number of cuttings that rooted and survived. A Fermate dust treatment was particularly effective in the protection of potato sets against rot and exerted a favourable influence upon the rooting of tamarisk and sequoia cuttings, but not upon cypress cuttings.

On the other hand, a copper oxide dust treatment increased the percentage of potato sets attacked by *Fusarium* spp., and Ceresan and Semesan dust treatments reduced the number of cypress cuttings that survived. Arasan Spergon, and Fermate do not appear to interfere with the natural healing of wounded tissue to the same extent as fungicides that contain copper and mercury, as measured by the loss of water from potato tissue cylinders. The inclusion of naphthalene acetic acid with Arasan, Spergon, and Fermate in treatments of cypress, tamarisk, and sequoia cuttings failed to exert a beneficial effect as measured by the numbers that rooted and survived. On the other hand, the inclusion of the hormone exerted a favourable effect upon holly cuttings.

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SIGN AND RANK TESTS[1]

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Recent developments in sign and rank tests have provided the research worker with statistical tools which enable him to conduct significance tests in a short time. On occasion the investigator may wish to make a quick appraisal of his results before employing the more elaborate statistical procedures. Sign and rank tests can then be used. Some of the tests require only mental arithmetic and such tests may be of use in the field.

Such rough-and-ready tests are available for testing significant differences between two or more means, for determining the degree of association between variables and for detecting trends or cycles in data. Examples of the various tests are given below.

Many of these rapid tests make use of the tables of the normal probability integral. In conducting significant tests it is important to distinguish between one-tail and two-tail tests. Whenever it is known the values from a treatment may equal or exceed but never be less than the control values the 95 per cent significant level, $t = 1.64$, is so chosen that 5 per cent of the area under the curve is in one tail of the curve only. If the treatment values may be above or below the control values, then a two-tailed test is used. The 95 per cent significant level, $t = 1.96$, is then chosen so that 2½ per cent of the area under the normal curve is in both tails of the curve.

SIGN TEST

This test (1) classifies each observation as a + or −. If N is the total number of observations made and S is the number of occurrences of the more numerous of the two signs then:

$$t = \frac{(2S - 1)}{\sqrt{N}} - \sqrt{N}$$

is approximately normally distributed and tables of the normal probability integral may be used to test the null hypothesis: that there is no significant departure of S from $\frac{N}{2}$.

Of 40 insects of one sex left to choose between two foods, A and B, 24 chose food A and 16 chose food B. Do these figures indicate a significant preference of one food over the other? The null hypothesis to be tested is that there is no indication of preference of one food over the other. Hence a two-tailed test is needed. Setting $N = 40$ and $S = 24$ we have;

$$t = \frac{(48 - 1)}{\sqrt{40}} - \sqrt{40} = 1.12.$$

Since this does not exceed 1.96, the 5 per cent level from the tables of the normal probability integral, the data do not disprove the null hypothesis.

Tables published by Mainland (3) give as the 95 per cent confidence limits for food A, 17 and 30 insects. Since 24 is between these limits the same conclusion is reached as by the sign test.

[1] Publication No. 302.
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RANK METHOD FOR COMPARISON OF TWO MEANS

If two means are based upon n_1 and n_2 observations then rank all the $N = n_1 + n_2$ observations . Compute the sum, R, of the ranks of one set of observations, n, usually the smaller of the two. Then compute $R' = R \pm 0.5$ using the sign which brings $2R'$ closer to $n(N + 1)$.

Under the null hypothesis that the two tests of observations came from the same population, we then test this hypothesis by means of;

$$t = \frac{2R' - n(N + 1)}{\sqrt{n(N + 1)(N - n)/3}}$$

where t is approximately normally distributed and tables of the normal probability integral may be used for probability levels (4). Since the original observations are replaced by their ranks the assumption made in the above test is that the n ranks are a random sample drawn without replacement from the integers from 1 to N.

Oviposition records for the parasite *Aptesis*, are given below. The treated group was fed honey while the control group was not.

Treated	Untreated
34	11
20	15
9	4
12	2
28	20
13	9
17	7

Rank N = 14 observations as follows:

2, 4, 7, 9, 9, 11, 12,
13, 15, 17, 20, 20, 28, 34,

and assign the ranks:

1, 2, 3, 4.5, 4.5, 6, 7,
8, 9, 10, 11.5, 11.5, 13, 14,

to them.

Then compute the sum of the ranks of the untreated group as follows:

$$R = 1 + 2 + 3 + 4.5 + 6 + 9 + 11.5 = 37.$$

Note that $R' = 37 + 0.5 = 37.5$ since the + sign will bring $2R'$ closer to $n(N + 1) = 7(15) = 105$. Substituting the above values in the formula we have,

$$t = \frac{2(37.5) - 105}{\sqrt{(7)(15)(7)/3}} = -1.91$$

Since the sum of the ranks of one group could conceivably be above or below the sum of the ranks of the other group a two-tailed test is used. Since t is not below -1.96, the 5 per cent level, the null hypothesis has not been disproved and on the basis of this test it is concluded that the oviposition records for the treated group are not significantly higher than those for the untreated group.

RANK TEST FOR COMPARISON OF ANY NUMBER OF GROUPS

The analysis of variance with the F test is usually used for making comparisons among several groups. If there are not too many observations in each group, a short test is available.

If there are n_i observations in the ith group where i = 1, 2, . . . k, and $\Sigma n_i = N$, then rank all N observations regardless of groups and assign a rank to each observation. The sum S_i of the n_i ranks in each group is obtained and;

$$\chi^2 = \frac{12\ \Sigma S_i^2/n_i}{N(N+1)} - 3(N+1)$$

is calculated. The above is approximately distributed as χ^2 with k − 1 degrees of freedom.

In the example below vitamin B_{12} production on Casamino Acid Medium A is given for 3 legume crops.

Lupine	Peas	Garden Beans
9.6 (7)	17.3 (6)	41.9 (3)
9.5 (8)	61.0 (1)	32.0 (5)
1.4 (12)	48.0 (2)	4.9 (9)
2.3 (11)	4.7 (10)	34.0 (4)
.4 (13)		

The ranks of the N = 13 observations are given in brackets above.

Computations necessary for insertion in the formula are given below;

	Lupine	Peas	Garden Beans
n_i	5	4	4
S_i	51	19	21
S^2	2601	361	441
S_i^2/n_i	520.20	90.25	110.25

$$\chi^2 = \frac{12(720.70)}{13 \times 14} - 3(14) = 5.51$$

with 2 degrees of freedom. Since this does not exceed the 5 per cent level for χ^2, 5.99, this rank test shows no significant differences in vitamin B_{12} production from the three crops based on the data submitted. The F test in an analysis of variance of the same data brings us to the same conclusion.

The superiority of the analysis of variance on data is that row, column, block effects, may be examined in addition to treatment effects.

SIGN TEST FOR TRENDS

In order to determine if there is an upward or downward trend in a series of observations, mark the successive differences + or −. These differences are not independent so the earlier sign test cannot be used. To determine if a greater number of the signs indicates a trend calculate:

$$t = \frac{2S - N}{\sqrt{(N+1)/3}}$$

where S is the number of the more numerous of the + or − signs and N is the total number of observations (not the total number of signs which is N − 1).

The t given above is approximatley normally distributed (5). To determine if the trend is in either direction then a two-tailed test is used and $t_{.05} = 1.96$ and $t_{.01} = 2.58$. To determine if movements in one direction are more numerous then a one-tailed distribution is used and $t_{.05} = 1.64$ and $t_{.01} = 2.33$.

The following day temperature records may be analysed to see if there is a significant upward trend in the data.

Temperature (°F.)

84.1	60.9 +	80.5 +
72.9 −	60.0 −	68.6 −
65.5 −	62.5 +	47.9 −
67.7 +	65.2 +	57.2 +
69.1 +	75.2 +	66.2 +
65.5 −	79.5 +	67.7 +
75.2 +	81.8 +	70.7 +
58.5 −	69.6 −	75.4 +
		69.1 −.

On substituting,

N = 25 and S = 15, we have:

$$t = \frac{30 - 25}{\sqrt{26/3}} = 1.70.$$

Since we wished to determine if the upward trend was the more numerous, 1.64 is the 5 per cent significance level in a one-tailed test and the upward trend is significant at that level.

SIGN TEST FOR GROUPING IN TRENDS

In order to determine if the grouping of the + and − signs is more than would be found in a random arrangement of N observations, the following test (8) may be made:

$$t = \frac{3g - 2N + 2.5}{\sqrt{(16N - 29)/10}}$$

where g is the number of groups in the + or − signs. Since too few groups indicate bunching a one-tailed test is made.

In the temperature data given previously there are 13 groups of like signs:

− −, ++, −, +, −, +, −, +++++, −, +, − −, +++++, −.

Substituting g = 13 and N = 25 in the above formula we have:

$$t = \frac{3(13) - 2(25) + 2.5}{\sqrt{[(16)(25) - 29]/10}} = -5.49$$

Since this is less than $t_{.01} = -2.33$, there is evidence of trends in the data.

This test would indicate if the grouping of signs, regardless of + or −, is more than would be expected in a random arrangement of observations and hence may be used to test the presence of cycles in the data.

RANK CORRELATION

If the observer has more confidence in the order of the results than in the absolute values obtained in determining the correlation between two variables, he may rank each of the variables separately and compute the ordinary cross-product correlation between the rank number of the values.

This computation simplifies to:

$$\rho = 1 - \frac{6\,\Sigma d^2}{N(N^2 - 1)}$$

where d is the difference in paired ranks and N is the number of pairs.

In order to test if the correlation is significant we compute:

$$t = \frac{6S' - (N^3 - N)}{N(N + 1)\sqrt{N-1}}$$

where N is the number of paired observations and $S' = S \pm 1$ where $S = \Sigma d^2$. Add or subtract 1 in such a way as to bring $6S'$ closer to $N^3 - N$. The t is approximately normally distributed (6) and usually the interest is in a two-tailed test.

The following tables of values give the average number of insects found in 9 sweeps per diem and the corresponding relative humidity values. Ranks are given in brackets.

Number of Insects	Humidity	d^2
1.5 (8)	49 (10)	4
3.5 (3.5)	76 (2)	2.25
3.0 (5.5)	58 (8.5)	9
5.0 (1)	64.5 (5)	16
4.5 (2)	62.5 (6)	16
3.5 (3.5)	74 (3)	.25
3.0 (5.5)	58 (8.5)	9
2.0 (7)	86 (1)	36
.5 (10)	61.5 (7)	9
1.0 (9)	65.5 (4)	25
		$S = 126.50.$

The rank correlation coefficient is first computed:

$$\rho = 1 - \frac{6(126.50)}{990} = +\ .23.$$

In order to determine if this correlation is significant compute $6S = 759$; this is less than 990 so 1 is added to S. Then $S' = 127.50$ and $6S' = 765$. Calculate:

$$t = \frac{765 - 9901}{10 \times 11\sqrt{9}} = -\ .68.$$

Since this is not below -1.96 the correlation as given by the above data between the average number of insects and the relative humidity is not significant.

A CORNER TEST FOR CORRELATION

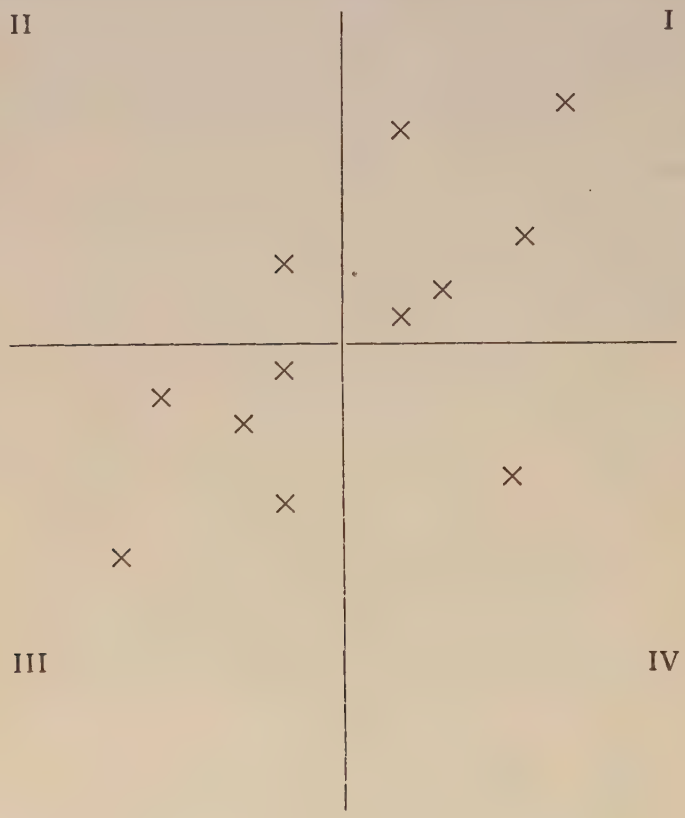
A quick test of association between two variables is provided by a quadrant sign test (7) which is non-parametric and gives special weight to extreme values of the variables. A median horizontal line is drawn through

the Y values and a median vertical line is drawn through the X values. The four quadrants so formed are labelled + − + −, the two quadrants in the upper right and lower left hand corner being +.

Starting at the right-hand side of the diagram, count along the observations above and below the X axis until forced to cross the Y axis. Attach a + sign to the number of observations in the upper right quadrant and a − sign to the number of observations in the lower right quadrant. Take the sum of the two numbers.

Repeat this process moving down from above, moving to the right from the left and moving up from below. The quadrant sum is the sum of the four terms thus obtained. The 5 per cent and 1 per cent levels of significance are given by at least 11 and 14 respectively, based on both tails of the distribution.

In the diagram below, the axes are the median lines of the data.



The quadrant sums are shown below:

Direction	*Quadrants*	*Values*	*Sum*
Right to left	I and IV	5−1	4
Top to bottom	I and II	5−1	4
Left to right	II and III	−1+5	4
Bottom to top	III and IV	5−1	4.

Since their sum is 16 and is above the 1 per cent level of significance there is a significant correlation between the two variables X and Y.

The authors of (7) give the treatment to be followed when an odd number of cases occur and when ties are present. An extension of this test to higher dimensions is also given.

GRAPHICAL PROCEDURES

One may resort to graphical methods in order to determine if the distribution follows the normal, Poisson or logarithmic curves.

Arithmetic probability paper has a linear scale and a cumulative normal scale. Plotting percentage cumulative frequencies of the normal curve on this paper yields a straight line, so that departure from normality may be recognized. Similar paper is available for the logistic curve and the discrete Poisson distribution.

Harding in (2) shows how to use the arithmetic probability paper for separating distributions which are polymodal and estimating the means and standard deviations of each of the contributing normal curves.

In addition to the familiar logarithmic and log-log graph papers used for transformations of variables, graph paper is available which transforms X to the inverse sine function. Straight lines then indicate whether this is a reasonable transformation.

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OBSERVATIONS ON THE OCCURRENCE AND CONTROL OF GREY MOULD ON GREENHOUSE CUCUMBERS[1]

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INTRODUCTION

Due to the demand for fresh vegetables during the winter season, the growing of greenhouse cucumbers has become an important part of the vegetable-growing industry in southwestern Ontario. In recent years, a large proportion of the 150 acres under glasshouse culture in Essex county has been devoted to the growing of this crop during the winter and spring months.

Coincident with the expansion in the greenhouse cucumber industry several diseases that affect the crop have appeared. Until 1947, grey mould on greenhouse cucumbers, caused by a *Botrytis* of the *cinerea* type, had been observed to be a disease of minor importance. However, in April of that year, the stem rot phase of this disease became epidemic in three of a group of five greenhouses on one farm at Leamington, Ontario. In one of the houses in which the disease was most severe, 30 per cent of the plants attacked were killed (3). The resultant crop loss was high. In the following year, the disease reappeared in the same series of greenhouses, and again a high percentage of the plants became infected. Similar outbreaks occurred in several other greenhouses in the Leamington area in that season. Since 1948, grey mould has occurred annually in many cucumber crops throughout much of the glasshouse area of Essex county and has come to be recognized as one of the most important diseases of the crop (4). Furthermore, the sudden appearance of this disease, which often remains unnoticed until a large number of plants have been attacked, many of which are destroyed within a few days, has made growers regard it with genuine concern. In contrast, the occurrence of a similar stem rot trouble, caused by *Sclerotinia sclerotiorum* (Lib) De Bary, has not paralleled that of the former disease but rather has remained a disease of relatively minor importance.

This paper presents observations on the effect of certain environmental and cultural conditions upon the development of grey mould. Effective means of control of this disease are also reported.

SYMPTOMS

Both the blossom blight and the stem rot phases of attack are commonly observed, and, of these, the latter is the more important. In the former, the first signs of the trouble are observed on the senescent and dead blossoms, where the fungus produces a fuzzy, greyish-brown fructification consisting of masses of conidiophores bearing numerous conidia. Frequently young fruits are invaded and rotted (*Figure 2*). Occasionally mature green fruits are found showing a small arrested lesion at the blossom end (*Figure 2*)

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Infections of the latter type originate in the female blossoms subsequent to fertilization. Stem lesions invariably begin at the nodes (*Figure 1*), girdle the stem, and ultimately kill the plants. Infection is initiated, not only upon blossoms, but also on petioles, tendrils, fruit peduncles and lateral branches that senesce and die as plant growth continues during the cropping season. Early in the season, the first stem lesions are usually found on the lower stem nodes, where maturation and death of plant organs occurs first. Later in the season, the upper nodes are attacked as frequently or even more so than the lower ones. Concomitant with lesion development is the early appearance of fungus fructifications producing prodigious numbers of conidia that are dispersed readily by air currents.

RELATION OF ENVIRONMENT TO DISEASE INCIDENCE

To establish infection, it is generally agreed that infection by *Botrytis* spp. requires the presence of some moribund or dead tissue coincident with a period of high humidity and low or moderate temperatures (1, 5, 12). It has been found (2, 10, 11, 14) that *Botrytis cinerea* grows best in culture or in various hosts at 72.5–77° F., but that it grows freely at 41° F., and slowly even at 32–35° F., thus meriting its recognition as a low temperature organism. Its growth is inhibited at 86°–95° F. Thomas (15) reported an epiphytotic of fruit and stem rot of tomatoes in a glasshouse where the temperature was held at 75°–80° F., with high humidity. Kadow, Anderson and Hopperstead (8) reported heavy losses in greenhouse cucumbers and tomatoes due to stem rots caused by a *Botrytis* sp. and *S. sclerotiorum*, in the spring of 1935 in the vicinity of Chicago, Illinois. During that period, damp cloudy weather prevailed. From observations of disease outbreaks caused by *B. cinerea* in outdoor ornamental and vegetable crops in California, Baker (1) reports that the fungus requires high humidity or free moisture to become aggressively parasitic and that it is favoured by a temperature considerably below 70° F.

Although it has been apparent to the author for several years that greenhouse humidity and temperature have influenced the incidence and severity of stem rot of cucumbers in southwestern Ontario, no experiments have been conducted to determine how these factors were operating. However, accurate records of numerous stem rot outbreaks in commercial greenhouses have yielded valuable information. In the last five years, the most serious outbreaks have occurred during April and early in May. In the years of 1947 and 1948, the high incidence of infection occurring during these two months coincided with periods of cool, damp, cloudy weather. More recently, however, although stem rot has been most prevalent during that period, serious outbreaks have occurred occasionally in March and also during the latter part of May. During March, April, and May, while greenhouse temperatures fluctuate appreciably in the daytime, night temperatures remain constant at about 65–70° F. During these months, humidity in the greenhouses usually remains high. In June, despite the frequent appearance of widespread incipient infections, *B. cinerea* has never been observed to cause serious losses. During June, greenhouse temperatures often rise to 80° and occasionally 90° F., or even remain above these temperatures for much of the daylight period. At the same time, when much ventilation can be provided in the greenhouses, the humidity is

appreciably lower than during the earlier spring months. Thus, during the growing season, greenhouse temperatures and humidities tend to be unfavourable for the development of the fungus only during the latter part of May or in June.

RELATION OF AGE AND CONDITION OF HOST TO DISEASE DEVELOPMENT

In Ontario greenhouses, cucumbers are usually planted in rows 24–40 inches apart, with a spacing of 18–24 inches between plants in the row. Plants are allowed to grow vertically to a height of 6–7 feet and are then trained along horizontal wires for a distance of 24–30 inches before they are "topped". Pruning is done at regular intervals to remove new growth beyond the formation of the first female blossom on the lateral branches.

The fact that stem rot outbreaks have occurred in cucumber crops only after the plants have reached a certain stage of development rather than at a certain period of the year, suggested that the ontogeny of the host is a highly important factor in the establishment of infection. To determine the time at which infection is initiated, the following experiment was carried out. Cucumber plants in a greenhouse held at a high humidity were inoculated by spraying them with conidial suspensions of *B. cinerea* at weekly intervals. Inoculations were begun when the plants were 16 inches high. Normal cultural practices were employed throughout the experiment.

Stem rot lesions were entirely absent until the cucumbers reached the topping stage. By that time, the lowest leaves and branches of some of the plants had become devoid of chlorophyll and had died. Before the fungus invaded healthy nodal and internodal tissue, it was observed to sporulate prolifically upon succulent dying and dead plant organs that remained attached to the cucumber stems at the nodes. After a heavy carpet of foliage had formed overhead, stem rot lesions were observed to develop at the nodes throughout the entire length of the stems. This same sequence of events, with respect to the occurrence and development of this disease, has been observed repeatedly in commercial crops.

Stem rot lesions have been observed to develop most rapidly and, resultant plant losses have been highest where growth has been lush and where, due to high humidity conditions, moribund tissues have remained succulent for long periods. On the other hand, under conditions of growth whereby the lower leaves have developed in light or moderate shade and where dying tissues have dried quickly, stem rot has never been observed to become serious.

CONTROL OF GREY MOULD

Prior to 1948, no attempts were made to prevent *Botrytis* infection in greenhouse cucumber crops in southwestern Ontario. On the appearance of the disease, an effort was made to lower the greenhouse humidity to check further disease development. In addition, sanitary measures consisting of the removal and destruction of infected tissues were carried out as thoroughly as possible. Although these measures afforded some belated control, attempts to lower greenhouse humidity frequently required considerable alteration in cultural practices that often resulted in lower production. Hence, other means of disease control seemed worthy of trial.

(*a*) *Soil Sterilization*

It has been found that the sterilization of the soil by steaming, although effective in the control of stem rot caused by *Sclerotinia sclerotiorum*, would not prevent grey mould from becoming established in new plantings. Early in the crop life, *B. cinerea* has many times been found to fruit abundantly upon plant organs that have been removed during the pruning operations and left lying on the surface of the manure mulch. Hence, manure mulches, usually applied subsequent to soil sterilization and after the young plants have been set in the greenhouses, may be regarded as a source of the grey mould fungus and an agency largely responsible for its introduction into greenhouses.

(*b*) *Application of fungicidal sprays*

Kadow, Anderson and Hopperstead (8) reported that sulphur sprays and dusts, as well as basic copper sulphate and Bordeaux mixture sprays, appeared to be effective in some tests in controlling Botrytis blossom blight and fruit rot in tomatoes and cucumbers. Results from later tests, however, led them to conclude that these treatments were not to be relied upon. They found that Bordeaux spray almost invariably injured cucumbers. In 1946, Klotz, Calavan and Zentmyer (9) reported that fermate was among the organic fungicides that would prevent the germination of *B. cinerea* conidia. More recently, Palmiter (13) and Holloman and Young (6) have reported fermate sprays to be highly effective in controlling infection in apples by *B. cinerea* and in gladiolus by *B. gladiolorum*, respectively.

In a series of greenhouses in which the disease became epidemic in the spring of 1947, sulphur and fixed copper (Copper A compound) sprays were applied. Neither of the fungicides gave effective control. The application of wettable sulphur sprays to cucumbers caused a "hardening" and stunting of the plants. Copper A compound sprays also occasionally caused mild injury to the foliage in the form of chlorosis.

In 1948, a section of a commercial greenhouse in which a stem rot outbreak had occurred was sprayed with fermate at the rate of 2 pounds to 100 gallons of water. Whereas many new infections continued to appear on the unsprayed plants, conspicuously fewer developed on the sprayed ones. No injurious effects upon cucumber growth were noted after five applications at weekly intervals.

A further test of the effectiveness of ferbam in preventing infection of cucumbers was made in the laboratory greenhouse in April, 1949. Six rows, each with eight cucumber plants per row, were sprayed with fermate at 8-day intervals until three applications were made. Six rows were left unsprayed as a check. Immediately after the residue of the first spray application had dried, conidia from Petri dish cultures of *B. cinerea* were distributed among the plants. This was done by removing the Petri dish covers and blowing the exposed masses of conidia in the direction of and among the plants. A second inoculation was made in the same manner 6 days later. This experiment was conducted under conditions favourable for infection by *B. cinerea*.

Although lesions were found on 84 per cent of the unsprayed plants, they were found on only 18 per cent of the plants in the sprayed block. Furthermore, whereas many of the infected plants in the unsprayed block

bore as many as 5 stem rot lesions, those infected in the sprayed area showed only one or rarely two lesions. A repetition of this experiment in 1950 yielded similar results. Additional observations of the effectiveness of ferbam sprays in the control of grey mould in commercial houses in 1949, 1950 and 1951 have substantiated these findings.

(*c*) *Induced Recovery of Infected Plants*

Although many growers have found that an outbreak of stem rot can be prevented by spraying the crop periodically with Ferbam, the importance of preventing premature death of already infected plants has also been realized. It is especially important to arrest the development of lesions that appear on the lower part of the cucumber stem early in the life of the plant.

Kadow *et al.* (8) reported excellent control of stem rot lesions in tomatoes and cucumbers by applying a Bordeaux paste to the lesions after they had become well established. The method was as follows: After the infected tissue was gouged out with a knife or stick, the cavity was filled with a thick paste made from Bordeaux mixture. The treatment, however, was found occasionally to cause injury in cucumbers, resulting in yellowed leaves.

An experiment was conducted in which a method of lesion treatment similar to that of Kadow *et al.* (8) was used. A planting of cucumbers in the laboratory greenhouse was heavily inoculated with *B. cinerea* conidia by the Petri plate dispersal method outlined above. When numerous rapidly developing stem lesions were present, they were tagged and treated as follows: A medium thin paste made by mixing Fermate with water was painted over many of the lesions. On approximately 50 per cent of the treated lesions, the infected tissues were scraped away with the thumbnail before the paste was applied. On the remainder of the treated lesions, the infected tissue was not removed (*Figure 1*). Care was taken to cover, not only the infected area, but also 1 or 2 cm. of apparently healthy stem tissue beyond the margin of the lesion. A number of lesions were left untreated as checks.

Twenty-four of the 50 untreated lesions had girdled the stems and killed the plants within a month. The remainder of the untreated lesions had enlarged considerably and the fungus had sporulated profusely on the infected tissues. In contrast, development was completely arrested in all except 8 of the 100 lesions painted with Fermate paste. Of this group only 4 plants died. No further lesion development was evident where the infected tissue was removed prior to the Fermate paste treatment. However, on a few of the treated lesions showing no development beyond the treated areas, the fungus was observed to grow through cracks in the dried crust of fermate and to sporulate sparingly.

In commercial greenhouse cucumber crops, the Fermate paste treatment has proven to be highly effective in arresting stem rot lesion development and consequently has reduced plant losses. Frequently, this treatment has prolonged the lives of severely infected cucumber plants for 2 or 3 months and has allowed them to produce apparently normal crops. This treatment was found to be non-injurious to cucumber plants.

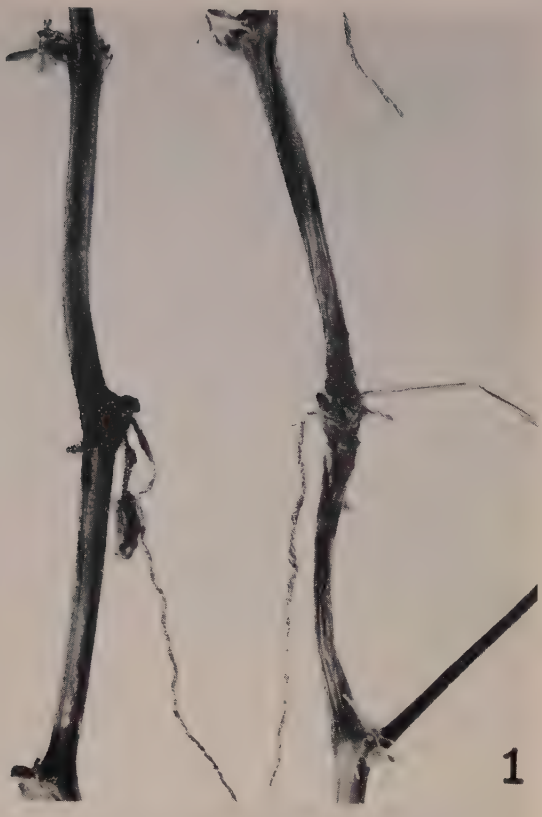

FIGURE 1. Portions of cucumber stems showing *Botrytis* lesions: *left* —lesion arrested by application of fermate paste to infected nodal tissue; *right*—check, non-treated lesion.

FIGURE 2. *Botrytis* rot of cucumber fruits. In these fruits infection became established upon blossoms subsequent to their fertilization.

DISCUSSION

Evidence has been presented to indicate that cultural and/or environmental greenhouse conditions may affect disease appearance and its subsequent development. Since greenhouse cucumber crops are forced, not only to bring them into early production, but also to maintain production at a high level, cultural practices involve more or less frequent applications of commercial fertilizers and manure mulches, along with frequent and heavy watering. Such practices often result in a luxuriant soft type of vegetative growth. The normal development of a heavy foliage at the upper parts of the plants causes yellowing and premature death of leaves and branches on the lower regions of the stems, especially in crowded stands where shading of the lower leaves results. This type of growth, in the presence of a high greenhouse humidity, invariably leads to the development of much moribund tissue of a succulent nature throughout the length of the cucumber stems. The latter substrate is a favourable one for the saprophytic development of *B. cinerea* prior to its invasion of adjacent healthy stem tissue. Moreover, cultural conditions that lead to the provision of suitable infection courts also create a moist local climate around the plants favourable for disease development. Thus, it would seem that environmental conditions and cultural practices that lead to a high greenhouse humidity, in addition to the production of much succulent moribund tissue, heighten the proneness of the cucumber crop to attack.

Since, at a certain stage of the ontogeny of the crop, conditions necessary for a disease outbreak are often fulfilled, the importance of applying Ferbam sprays to prevent infection at that time has been demonstrated. The ability to induce recovery of severely infected plants and thereby reduce crop losses by painting the lesions with a Fermate paste has also been demonstrated.

The question why *Botrytis* stem rot of greenhouse cucumbers has shown increased prevalence in recent years presents itself. It is noteworthy that this disease has only assumed major importance since the Burpee hybrid variety has been substituted for older varieties. This variety is highly productive and a vigorous type which, under ordinary greenhouse conditions, has produced a heavier foliage than that of varieties previously grown. As a consequence of this heavy vegetative growth habit, when the normal planting distance is used, the lowest leaves and branches of the hybrid variety have been found to die earlier than those of other varieties, hence providing suitable infection courts for the fungus early in the life of the crop. Besides, the increased prevalence of stem rot may also be due, in part, to the planting of many greenhouse crops in Ontario earlier now than a few years ago. With earlier planting there is a greater likelihood that the period when the host is most vulnerable to attack will coincide with greenhouse temperature and humidity favourable for disease development.

SUMMARY

In recent years stem canker and fruit rot, caused by *Botrytis cinerea*, has been prevalent and destructive in many greenhouse cucumber crops in southwestern Ontario.

Moribund plant organs, including not only blossoms but also tendrils, petioles, lateral branches and fruit pedicels, have been found to serve as infection courts for the fungus prior to its invasion of healthy tissues. When established in a greenhouse, the disease has been observed to spread rapidly. In the presence of high greenhouse humidity combined with a soft vegetative growth, lesions originating at the nodes rapidly girdled the stems and killed the plants.

The application of Fermate sprays when the crop reached the susceptible stage was found to reduce substantially the percentage of infections. Effective control of stem lesions was obtained by covering the diseased areas with a thin paste of Fermate. Stem treatments and periodic sprays with Fermate were found to be non-injurious to the cucumber crop.

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A COMPARISON OF CRUMBLED PELLETS AND MASH, WITH AND WITHOUT PROCAINE PENICILLIN, ON GROWTH OF TURKEY POULTS[1]

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Commercial poult starters supplemented with antibiotics are at present available in pelleted, crumbled and mash form. The crumbled form is produced by pelleting the mash and recrushing these pellets to produce an angular particle approximately one-eighth inch in diameter. This has given rise to the question of the possible advantages of texture for poult growth, and whether or not the pelleting process destroys the growth-stimulating activity of the antibiotic. Robertson (5) reported that crumbles gave a greater growth response for poults than did mash, and that the difference was more marked with floor brooded poults than with those in batteries. Ziegenhagen *et al.* (6) found that rate of growth of poults was increased significantly for the first eight weeks through feeding of crumbles. These authors reported a tendency toward cannibalism in the groups fed the granular feed. Blakely and co-workers, using commercial mash and crumbles said to be of the same formulation, reported[5] that the crumbled form of turkey starter gave significantly better growth than the mash form, and that a mixture of crumbles and mash gave a growth rate which was intermediate between the crumbles and the mash. No indication of cannibalism was noted on any of their diets. McGinnis (3), Davis and Briggs (2), MacGregor *et al.*[6] and others have demonstrated that poult growth is stimulated by the addition of procaine penicillin to the starter mash. No data are available on the stability of procaine penicillin under the conditions prevailing during the pelleting process. Patrick (4) failed to produce a growth response when a vitamin B_{12} supplement was added to a soybean diet for poults. Atkinson and Couch (1) reported that, under the conditions of their experiment, turkey poults did not require a dietary source of vitamin B_{12} when fed a yellow corn, soybean oil meal diet. MacGregor, Anderson and Blakely[6] reported that the addition of a vitamin B_{12} supplement to a poult starter mash did not produce a growth response, but that the addition of an antibiotic-vitamin B_{12} supplement did produce a growth response. Their control diet was identical with that used in the present experiment.

The present experiment was undertaken at this laboratory to determine the effect on poult growth of pelleting and recrumbling a turkey poult starter with and without procaine penicillin, and to determine the effect of pelleting on the growth-promoting activity of this antibiotic.

[1] Contribution from the Experimental Farms Service, Department of Agriculture, Canada.
[2] Officer-in-Charge, Turkey Research Laboratory.
[3] Graduate Assistant, Poultry.
[4] Research Officer, Turkey Nutrition.
[5] Annual Report, Dominion Experimental Station, Swift Current, Saskatchewan, Canada. 1950 (*unpublished*).
[6] Annual Report. Dominion Experimental Station, Swift Current, Saskatchewan, Canada. 1951 (*unpublished*).

PROCEDURE

Two hundred and seventy day-old Broad Breasted Bronze turkey poults were distributed, on the basis of weight, into five groups of 54 poults each. They were housed in wire-floored battery brooders in a room in which the temperature was thermostatically controlled. Body-weight and feed consumption data were recorded weekly. Morbidity and mortality were recorded at the time of occurrence. The basal diet consisted of: ground barley 10; ground oat groats 15; pulverized whole oats 5; ground wheat 14; wheat bran 5; dehydrated ground cereal grasses 5; dried brewers' yeast 3; fish meal 5; milk powder 5; horse-meat meal 5; soybean oil meal 23.5; bone meal 1; ground limestone 1.6; salt 0.5; fish oil (1200A–200D) 1; choline chloride (25% mix) 0.2; manganese sulphate 0.02; potassium iodide 0.0005; riboflavin (0.001% mix) 0.15. This mash was of medium-coarse texture.

All mashes were mixed in this laboratory, but the pelleting and crumbling processes were undertaken through courtesy of the Saskatchewan Federated Co-Operatives, Saskatoon, Saskatchewan.

Details of the pelleting procedure were supplied by the Federated Co-Operatives. Steam at 80 p.s.i. was used at the pellet mill. This heat plus the pressure applied at the rollers dried the processed feed to within 1 per cent of the original moisture content of the mash. Pellets 3/32 inch in diameter were produced. They were then passed through the cooler to the crumbelizer and then to overhead bins for sacking. Temperature of the feed leaving the conditioning chamber was 45° C. There was no loss in temperature as the material passed to the rollers and dies. Temperature of the pellets leaving the pellet mill was 55° C.

The penicillin added to these diets was in the form of a commercial antibiotic-vitamin B_{12} supplement.

TABLE 1.—THE EFFECT OF DIET TEXTURE AND THE PRESENCE OR ABSENCE OF PROCAINE PENICILLIN ON POULT GROWTH

Diet No.	Dietary Treatment	Sex	Body Weight	
			4 wk.	8 wk.
			gm.	gm.
34-A	Mash. No antibiotic	M	378	1635
		F	308	1331
		M & F	343	1483
34-B	Mash + 4.41 p.p.m. procaine penicillin	M	590	2102
		F	557	1910
		M & F	573.5	2006
34-C	Crumbles. No antibiotic	M	349	1444
		F	370	1422
		M & F	359.5	1433
34-D	Crumbles + 4.41 p.p.m. procaine penicillin (before pelleting)	M	678	2334
		F	590	1922
		M & F	634	2128
34-E	Crumbles + 4.41 p.p.m. procaine penicillin (after pelleting)	M	675	2314
		F	579	1894
		M & F	627	2104

RESULTS AND DISCUSSION

The effects of diet texture and the presence or absence of procaine penicillin on poult growth are shown in Table 1.

Analysis of variance of body weight data at 4 and 8 weeks of age indicates that procaine penicillin caused a highly significant increase in growth, whether added to the mash (diet 34-B), added to the mixture before pelleting and crumbling (diet 34-D), or added after the pelleting and crumbling process (diet 34–E). The pelleting operation did not appear to decrease the growth-stimulating activity of the antibiotic. Pelleting had no significant effect on growth of poults receiving no penicillin, but did have a significant effect on the growth of males in the groups receiving the antibiotic.

Unusually high mortality occurred in some of the treatments during the first four weeks. The cause of this high mortality was not determined. It is of interest to note in passing, however, that the groups receiving the diets containing no antibiotic suffered the greatest losses.

A perosis type of leg weakness occurred in all groups. Procaine penicillin in the diet did not prevent the occurrence of this condition.

SUMMARY AND CONCLUSIONS

Five groups of 54 poults each were used to determine the effect on growth of the texture of a starter mash and of the addition of procaine penicillin at 4.41 p.p.m., before and after pelleting.

Procaine penicillin resulted in a highly significant growth increase whether added to the mash or to the crumbled form of diet. The pelleting and crumbling process had no effect on the growth-promoting activity of the procaine penicillin.

Feeding the diet in crumbled (recrushed pellet) form had no significant effect on growth when neither diet contained penicillin. There was a significant increase in the body-weights of males fed the granular type diet when both diets contained penicillin.

Leg weakness was not prevented by the addition of penicillin to the diet.

ACKNOWLEDGMENTS

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A NOTE ON THE PODSOLIZATION PROCESS

In a recent communication (1) it was reported that aqueous extracts, prepared in the laboratory from undecomposed leaves and bark of New Zealand kauri, and from pine needles, dissolved hydrous oxides of iron and aluminium, reduced ferric iron, and formed complexes with ferrous ions. The conclusion was drawn that natural leachates from undecomposed coniferous litter provided all conditions essential for podsolic translocation of sesquihydroxides.

In investigations begun in 1947 similar results in respect of solution of iron and aluminium (2), and of reduction and complexing of iron (3) have been obtained by workers in this laboratory employing, primarily, leachates produced in the field by natural rainfall from decomposing deciduous leaves and coniferous needles. Leaf and leachate collections have been described (4). Solutions obtained by aqueous extraction of ground mature leaves collected as soon as feasible after leaf-fall also have been studied. The natural leachates and the laboratory extracts have, in general, shown similar characteristics except that extracts are more concentrated, apparently more complex, and as might be expected, show greater reducing power and iron uptake than leachates.

Titrations with ferric alum solutions have shown that the capacities of these solutions for reduction of ferric ion decrease sharply with pH, becoming zero at about pH 3, and that ferrous iron reactive with ferricyanide appears in them well before maximum reducing power has been exerted.

The uptake of iron from cation-exchange resins by leachates and extracts has been determined. In this way complications involved when iron salts are added to such solutions were avoided. Extracts from leaves of four species of deciduous tree, when allowed to flow over the cation-exchanger Dowex 50 previously saturated with ferric ion removed varying amounts of iron. The major part, 80 per cent or more, of the iron in the effluents reacted with α, α'-dipyridyl. The rates of reaction with this reagent differed with effluents obtained from leaves of different species and, in general, a period of about 8 hours was required to ensure completion. Leachates treated similarly showed similar behaviour in these respects. The slow rate of reaction of the ferrous iron in the effluents with dipyridyl, and the non-appearance of ferrous ion reactive to ferricyanide during the early part of the titrations with ferric alum, provide strong evidence that complex formation takes place between ferrous ions and components of the leachates and extracts. Results of conductometric titrations with ferric salt solution and of electrophoretic experiments with leachates and extracts enriched with iron by cation exchange provide further evidence for the formation of such complexes. The dipyridyl-reactive iron in iron-enriched leachates was found to be stable to aeration with a stream of oxygen for 24 hours.

Passage of iron-enriched extracts over cation-exchange resins saturated with hydrogen, aluminium and calcium ions respectively resulted in replacement of decreasing amounts of iron in that order. This behaviour may be

related to the variable thicknesses of the horizons of eluviation and deposition of iron in various podsolic soils. These horizons are, in general, thinner in the strongly acidic podsol soils than in the gray-brown podsolic soils which are more highly saturated with calcium.

A more detailed account of these experiments is being prepared for publication.

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